Biomarkers in Osteoarthritis

by

Oliver Patrick Stannus, BE(Hons)

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Supervisors **Associate Professor Changhai Ding**

Professor Graeme Jones

Doctor Danchi Jiang

Doctor Stephen Quinn

Statement of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Statement of Co-Authorship

This thesis includes papers for which Oliver Stannus (OS) was not the sole author. OS was the lead in the research of each manuscript; however, he was assisted by the co-authors, whose contributions are detailed below.

Chapter 4:

Stannus O, Jones G, Quinn S, Cicuttini F, Dore D, Ding C. The association between leptin, interleukin-6 and hip radiographic osteoarthritis in older people: A cross-sectional study. Arthritis Research & Therapy. 2010;12(3): R95.

The contribution of each author:

OS was responsible for data management and cleaning, carried out analysis and interpretation of data, prepared the initial manuscript draft and completed manuscript revisions.

GJ and FC designed and carried out the study planning, participated in analysis and interpretation of data and critically revised the manuscript.

QS participated in analysis and interpretation of data and critically revised the manuscript.

DD critically revised the manuscript.

CD designed and carried out the study planning, participated in analysis and interpretation of data, assisted with the initial manuscript draft and critically revised the manuscript.

Chapter 5:

Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J, Ding C. Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. Osteoarthritis Cartilage. 2010;18:1441-7.

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QS participated in analysis and interpretation of data and critically revised the manuscript.

VP and JB carried out data collection and critically revised the manuscript.

CD designed and carried out the study planning, was responsible for data collection, participated in analysis and interpretation of data, assisted with the initial manuscript draft and critically revised the manuscript.

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GJ and FC designed and carried out the study planning, participated in analysis and interpretation of data and critically revised the manuscript.

LB participated in analysis and interpretation of data and critically revised the manuscript.

CD designed and carried out the study planning, was responsible for data collection, participated in analysis and interpretation of data, assisted with the initial manuscript draft and critically revised the manuscript.

Chapter 7:

Carnes J, Stannus O, Cicuttini F, Ding C, Jones G. Knee Cartilage Defects in a Sample of Older Adults: Natural History, Clinical Significance and Factors Influencing Change over 2.9 years. Osteoarthritis and Cartilage 2012 Sep 6. [Epub ahead of print].

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FC designed and carried out the study planning, participated in analysis and interpretation of data and critically revised the manuscript.

GJ designed and carried out the study planning, participated in analysis and interpretation of data, assisted with the initial manuscript draft, critically revised the manuscript and completed manuscript revisions.

Chapter 8:

Cross-sectional and longitudinal associations between circulating leptin and knee cartilage thickness in older adults. Stannus O, Cao Y, Jones G, Blizzard L, Antony B, Ding C. Manuscript submitted to Annals of the Rheumatic Diseases

The contribution of each author:

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GJ designed and carried out the study planning, participated in analysis and interpretation of data and critically revised the manuscript.

LB participated in analysis and interpretation of data and critically revised the manuscript.

BA critically revised the manuscript.

CD designed and carried out the study planning, participated in analysis and interpretation of data, assisted with the initial manuscript draft and critically revised the manuscript.

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OS was responsible for data collection, data management and cleaning, carried out analysis and interpretation of data and prepared the initial manuscript draft.

DJ participated in analysis and interpretation of data and critically revised the manuscript.

FC designed and carried out the study planning, participated in analysis and interpretation of data and critically revised the manuscript.

YC critically revised the manuscript.

CD designed and carried out the study planning, participated in analysis and interpretation of data, assisted with the initial manuscript draft and critically revised the manuscript.

(Signed) _____ (Date) _____

Oliver Stannus (Candidate)

(Signed) _____ (Date) _____

Changhai Ding (Primary supervisor)

Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

Abstract

Osteoarthritis (OA) is a multifactorial disease of the joints, common among older adults, which can lead to pain, impaired function and reduced quality of life. This thesis aims to investigate the associations and predictive value of various hormonal, inflammatory and imaging biomarkers with OA outcomes in population-based studies of people with and without prevalent OA.

Two population samples were used in this thesis. The first group was a population-based sample of older adults aged 50-80 years (mean age: 62 years; 51% female). Followup measurements were conducted 2.7 (2.6-3.3) years later and again for questionnaire data 5.0 (5.3-6.8) years later. Magnetic resonance imaging (MRI) on the right knees was undertaken at baseline and first followup: knee cartilage volume, tibial bone area, cartilage defects and bone marrow lesions (BMLs) were measured or scored; cartilage mean T1 signal intensity and thickness were measured by semi-automated software. Baseline knee and hip x-rays were scored for joint space narrowing (JSN) and osteophytes. Serum leptin and cytokine levels were measured by immunoassay at baseline and first followup. Body morphometry was measured at baseline. Fat and lean mass measures were measured at baseline using dual-energy x-ray absorptiometry (DXA). Knee pain was assessed by questionnaires (WOMAC, Western Ontario and McMasters Osteoarthritis Index) at all timepoints.

The second group was a population-based sample of younger adults aged 26-51 (mean age 41; 64% female). Anthropometric, x-ray and MRI-derived scores and measures were obtained as in the first group. Urinary C-terminal crosslinking telopeptide of type II collagen (U-CTX-II) was measured by measured by immunoassay.

This thesis consists of 6 studies. In the first study, in older adults, circulating levels of both leptin and interleukin-6 (IL-6) were associated with hip JSN in both sexes and females respectively, independently of BMI. Adiposity was associated with hip JSN, but not after adjustment for leptin.

In the second study, baseline levels of both IL-6 and tumor necrosis factor alpha (TNF-α) were associated with medial tibiofemoral knee JSN. Baseline IL-6, change in IL-6 and change in TNF-α were associated with cartilage volume loss.

In the third study, in older adults, baseline or change over 2.9 years in circulating levels of high sensitivity C-reactive protein (hs-CRP), IL-6 and TNF-α were associated with change over 5 years in sub-scale or total WOMAC knee pain.

In the fourth study, higher leptin in older adults was significantly associated with lower femoral, tibial and patellar cartilage thickness. Fat measures were negatively associated with cartilage thickness, largely mediated by leptin. Baseline and change in leptin were associated with medial tibial cartilage thickness loss.

In the fifth study, knee cartilage defects in older adults were found to be common, not likely to regress, and to predict cartilage volume loss and risk of knee replacement.

In the final study, mean T1 MRI signal intensity of cartilage was negatively associated with BMI and same-region cartilage defects in younger and older adults; with U-CTX-II in younger adults; and with JSN and osteophytes in older adults at various sites. It predicted cartilage thickness loss over 2.7 years in older adults.

In conclusion, inflammatory and metabolic factors may play important roles in aetiology of cartilage loss and/or symptoms in OA. Cartilage defects predict cartilage loss and risk of knee replacement, and mean T1 MRI signal intensity of cartilage predicts loss of cartilage thickness. All these are potential biomarkers for OA at risk of development or progression, and thus possible targets for intervention.

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Publications Arising from the Thesis

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Chapter 5: Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J, Ding C. Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. *Osteoarthritis and Cartilage. 2010;18:1441-7.*

Chapter 6: Stannus O, Jones G, Blizzard L, Cicuttini F, Ding C. Associations between serum levels of inflammatory markers and change in knee pain over 5 years in older adults: a prospective cohort study. *Annals of the Rheumatic Diseases 2013; 72(4):535-40*

Chapter 7: Carnes J, Stannus O, Cicuttini F, Ding C, Jones G. Knee Cartilage Defects in a Sample of Older Adults: Natural History, Clinical Significance and Factors Influencing Change over 2.9 years. *Osteoarthritis and Cartilage 2012;20:1541-7.*

Chapter 8: Stannus O, Cao Y, Jones G, Blizzard L, Antony B, Ding C. Cross-sectional and longitudinal associations between circulating leptin and knee cartilage thickness in older adults. *Annals of the Rheumatic Diseases 2013 Sep 27 [e-pub ahead of print]*

Chapter 9: Stannus O, Jiang D, Cicuttini F, Cao Y, Ding C. Cartilage signal intensity on T1 weighted MRI: association with risk factors and measures of knee osteoarthritis. *Manuscript submitted to Clinical Rheumatology.*

Other Publications

Antony B, Jones G, Stannus O, Blizzard L, Ding C. Body fat predicts an increase and limb muscle strength predicts a decrease in leptin in older adults over 2.6 years. *Clinical Endocrinology 2012*. [Epub ahead of print]

Antony B, Ding C, Stannus O, Cicuttini F, Jones G. Association of baseline knee bone size, cartilage volume, and body mass index with knee cartilage loss over time: A longitudinal study in younger or middle-aged adults. *Journal of Rheumatology 2011; 38(9):1973-80*

Ding C, Stannus O, Cicuttini F, Antony B, Jones G. Body fat is associated with increased and lean mass with decreased knee cartilage loss in older adults: a prospective cohort study. *International Journal of Obesity 21 August 2012*. [Epub ahead of print]

Cao Y, Stannus O.P., Aitken D, Cicuttini F, Antony B, Jones G, Ding C. Cross-sectional and longitudinal associations between systemic, subchondral bone mineral density and knee cartilage thickness in older adults with or without radiographic osteoarthritis. *Annals of the Rheumatic Diseases July 2013.* [Epub ahead of print]

Scientific Presentations and Awards Arising from the Thesis

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Wellington, New Zealand
(**Oral presentation**)

2009 World Congress on Osteoarthritis (Osteoarthritis Research Society International Annual Meeting)
Serum levels of inflammatory markers, knee radiographic osteoarthritis, and knee cartilage loss in older adults
Montreal, Canada
(Oral presentation - presented by co-author)

2009 World Congress on Osteoarthritis (Osteoarthritis Research Society International Annual Meeting)
The associations between leptin, interleukin-6 and hip radiographic osteoarthritis in older people
Montreal, Canada
(Oral presentation - presented by co-author)

2011 Australian Rheumatology Association Annual Scientific Meeting
Cartilage signal intensity on MRI: association with body mass index, cartilage defects and type II collagen breakdown
Brisbane, Australia
(Poster presentation - **Best basic science poster prize**)

2011 World Congress on Osteoarthritis (Osteoarthritis Research Society International Annual Meeting)
Inflammatory biomarkers are predictive of increases in knee pain over 5 years in older adults
San Diego, USA

2011 Australian Rheumatology Association Annual Scientific Meeting
Inflammatory biomarkers are predictive of increases in knee pain over 5 years in older adults
Brisbane, Australia
(Poster presentation)

2011 Osteoarthritis Research Society International Imaging Meeting
Cartilage signal intensity on MRI: association with body mass index, cartilage defects and type II collagen breakdown
Salzburg, Austria.
(Poster presentation)

2012 American College of Rheumatology Annual Meeting
Cross-sectional and longitudinal associations between circulating leptin and knee cartilage thickness in older adults
Washington DC, USA
(**Oral presentation** - presented by co-author)

List of Abbreviations

2D	two-dimensional
BIPED	burden, investigative, prognostic, efficacy or diagnostic
BLOKS	Boston–Leeds osteoarthritis score
BME	bone marrow edema
BMI	body mass index
BML	bone marrow lesion
CI	confidence interval
COMP	cartilage oligomeric protein
CRP	C-reactive protein
CTX-II	C-terminal crosslinking telopeptide of type II collagen
CV	coefficient of variation
DALY	disability-adjusted life year
dGEMRIC	delayed gadolinium-enhanced MRI of cartilage
DMOAD	disease modifying anti-osteoarthritis drug
DXA	dual energy x-ray absorpiometry
FSE	fast spin echo
GEE	generalised estimating equation
GRE	gradient recall echo
HOAMS	hip osteoarthritis MRI scoring system
hs-CRP	high-sensitivity C-reactive protein
IFN-γ	interferon gamma
IL-1	interleukin 1
IL1-β	interleukin 1-beta
IL-6	interleukin 6
JSN	joint space narrowing
KCVS	knee cartilage volume study
KL	Kellgren–Lawrence
KOSS	knee osteoarthritis scoring system
MMP	matrix-metalloproteinase
MOAKS	MRI knee osteoarthritis score
MRI	magnetic resonance imaging
NO	nitric oxide

NOS	nitric oxide synthase
NSAID	non-steroidal anti-inflammatory drug
OA	osteoarthritis
OARSI	osteoarthritis research society international
OHA-MRI	Oslo hand osteoarthritis MRI score
OP	osteophyte
QALY	quality-adjusted life year
RA	rheumatoid arthritis
ROA	radiographic osteoarthritis
ROI	region of interest
SD	standard deviation
TASOAC	Tasmanian older adult cohort
THR	total hip replacement
TJR	total joint replacement
TKR	total knee replacement
TNF-α	tumor necrosis factor alpha
uCTX-II	urinary C-terminal crosslinking telopeptide of type II collagen
WHR	waist–hip ratio
WOMAC	Western Ontario and McMasters pain questionnaire
WORMS	whole organ magnetic resonance imaging score

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Synopsis

OA is a common disease in older adulthood and a cause of reduced function and quality of life. It is generally described as multifactorial, being a complex disease involving multiple biological pathways and processes. The end result is degradation and loss of articular cartilage. However, many tissues are involved in the aetiology of this process, including the synovium, menisci, subchondral bone, ligaments and surrounding muscles. Many risk factors for OA have been identified, including age, being female and obesity; however, much of the aetiology and disease process is not well understood. Current evidence strongly suggests OA has a stage of early disease prior to radiographic changes, in which biochemical and biomechanical changes provide an environment for altered cartilage homeostasis, which can lead to cartilage defects and other early pathological changes detectable on MRI. This thesis examines the descriptive and predictive value of various hormonal, inflammatory and imaging biomarkers with knee and hip OA outcome measures in population-based studies of people with and without prevalent OA. Specifically, it examines possible relationships between of leptin, inflammatory biomarkers, mean cartilage T1 signal intensity and cartilage defects with OA risk factors, severity and progression. The importance of these measures in defining those at risk of OA from these causal pathways is discussed, as well as the opportunities for therapeutic intervention. This synopsis summarises the content of each chapter.

Chapter 1 gives an introduction to OA, including currently understood aetiology and risk factors. An overview of current methodologies for quantifying OA presence and progression is provided, examining clinical and research measures. A discussion of biomarkers of OA severity and progression risk is given.

Chapter 2 lists the research questions to be addressed in this thesis.

Chapter 3 describes the Tasmanian Older Adult Cohort (TASOAC) and Knee Cartilage Volume (KCV) studies, detailing both study populations, protocols for measurements of factors which are common to multiple chapters in this thesis. Additional factors which are unique to individual chapters are described in more detail in the methodology section of respective chapters.

Chapter 4 describes the cross-sectional associations between leptin, IL-6 and hip radiographic osteoarthritis in older people. A cross-sectional sample of 193 randomly selected subjects (mean 63 years, range 52-78, 48% female) were studied. Hip ROA including joint space narrowing (JSN) and osteophytes was determined by anteroposterior x-ray. Serum levels of leptin and interleukin IL-6 were measured by radioimmunoassay. Fat mass was measured by dual energy x-ray absorptiometry (DXA). Body mass index (BMI) and waist to hip ratio (WHR) were calculated. In multivariable analysis, hip JSN was associated with serum levels of leptin in the whole sample ($\beta = 0.046$ per μg/litre, $p = 0.024$ for superior; $\beta = 0.068$ per μg/litre, $p = 0.004$ for axial compartment) and IL-6 only in females ($\beta = 0.241$ per pg/ml, $p = 0.002$ for superior; $\beta = 0.239$ per pg/ml, $p = 0.001$ for axial compartment). The positive associations between body composition measures (BMI, WHR, percentage total fat mass and percentage trunk fat mass) and hip JSN in females became non-significant after adjustment for leptin but not for IL-6. There were no significant associations between leptin, IL-6 and the presence or severity of osteophytes. In conclusion, this study suggests that metabolic and inflammatory mechanisms may play a role in the aetiology of hip OA and that the associations between body composition and hip JSN are mediated by leptin, particularly in females.

Chapter 5 describes the cross-sectional and longitudinal associations of both IL-6 and tumor necrosis factor alpha with knee radiographic osteoarthritis and knee cartilage loss in older adults. A total of 172 randomly selected subjects (mean 63 years, range 52-78, 47% female) were studied at baseline and approximately 3 (range 2.6 to 3.3) years later. IL-6 and TNF-α were assessed by radioimmunoassay. T1-weighted fat-suppressed MRI of the right knee was performed at baseline and followup to determine knee cartilage volume. Knee radiographic osteoarthritis (ROA) of both knees was assessed at baseline. At baseline, quartiles of IL-6 and TNF-α were associated with increased prevalence of medial tibiofemoral joint space narrowing (OARSI grade ≥1) in multivariate analyses (OR: 1.42 and 1.47 per quartile, respectively, both $P<0.05$). Longitudinally, baseline IL-6 predicted loss of both medial and lateral tibial cartilage volume (β: -1.19% and -1.35% per annum per quartile, $P<0.05$ and $P<0.01$, respectively), independently of TNF-α. Change in IL-6 was associated with increased loss of medial and lateral tibial cartilage volume (β: -1.18% and -1.06% per annum per quartile, both $P<0.05$) and change in TNF-α was also negatively associated with change in medial cartilage volume (β: -1.27% per annum per quartile, $P<0.05$). In conclusion, serum levels of IL-6 and TNF-α are associated with knee cartilage

loss in older people suggesting low level inflammation plays a role in the pathogenesis of knee OA.

Chapter 6 determines the associations between inflammatory biomarkers and changes in knee pain over 5 years. A total of 149 randomly selected subjects (mean 63 years, range 52-78; 46% female) were studied. Serum levels of C-reactive protein (hs-CRP), TNF-α and IL-6 were measured at baseline and 2.7 years later. Knee pain was recorded using the WOMAC questionnaire at baseline and 5 years later. Knee ROA of both knees was assessed at baseline, and knee bone marrow lesions, joint effusion and cartilage defects were determined using T1- or T2-weighted fat saturated MRI. After adjustment for confounding variables, baseline hs-CRP was positively associated with change in total knee pain (β=0.33 per mg/L, P=0.032), as well as change in the pain at night in bed (β=0.12 per mL/pg, P=0.010) and while sitting/lying (β=0.12 per mL/pg, P=0.002). Change in hs-CRP was also associated with change in knee pain at night and when sitting/lying (both $P<0.05$). Baseline TNF-α and IL-6 were associated with change in pain while standing (β=0.06 per mL/pg, P=0.033; β=0.16 per mL/pg, P=0.035, respectively), and change in TNF-α was positively associated with change in total knee pain (β=0.66 mL/pg, p=0.020) and change in pain while standing (β=0.26 mL/pg, p=0.002). Adjustment for ROA or MRI-detected structural abnormalities led to no or minor attenuation of these associations. In conclusion, systemic inflammation is an independent predictor of worsening knee pain over 5 years.

Chapter 7 describes the natural history of knee cartilage defects, and their relationship to cartilage volume loss and risk of knee replacement in a longitudinal study of older adults. A total of 395 randomly selected older adults (mean age 62.7 years) had magnetic resonance imaging of their right knee at baseline and approximately 2.9 years later to determine cartilage defect grade (0-4), cartilage volume, medial and lateral tibial bone size, and presence of BMLs. Height, weight, BMI and ROA were measured by standard protocols. At baseline higher grade cartilage defects (grade ≥ 2) were significantly associated with age, BMI, lateral tibial bone size, BMLs, and ROA. Over 2.9 years, the average defect score increased significantly in all compartments; however, the majority of defects remained stable and regression of defects was rare. Baseline factors associated with increase in defect score over 2.9 years were ROA, tibial bone size, BMI and being female. In multivariate analysis, baseline cartilage defect grade predicted cartilage volume loss at

the medial tibia, lateral tibia and patella over 2.9 years (β=-1.78% to -1.27% per annum per 1 grade increase, $p < 0.05$ for all comparisons), and risk of knee replacement over 5 years (OR = 1.73 per 1 grade increase, p=0.001). In conclusion, knee cartilage defects in older adults are common but less likely to regress than in younger life. They independently predict cartilage volume loss and risk of knee replacement, suggesting they are potential targets for intervention.

Chapter 8 describes the cross-sectional and longitudinal associations between serum leptin levels and knee cartilage thickness in older adults. A prospective cohort of 163 randomly selected subjects (mean 63 years, range 52–78, 46% female) were studied. Knee cartilage thickness at medial tibial, lateral tibial, femoral and patellar sites was determined using T1-weighted fat suppressed MRI. Serum leptin levels were measured by radioimmunoassay. ROA, body fat (%), trunk fat (%), weight and height were measured, and BMI was calculated. Cross-sectionally, serum levels of leptin were negatively associated with femoral (β: -0.013, 95% CI: -0.022, -0.003), medial tibial (β: -0.009, 95% CI: -0.018, 0.001), lateral tibial (β: -0.012, 95% CI: -0.021, -0.003) and patellar (β: -0.014, 95% CI: -0.026, -0.002) cartilage thickness after adjustment for covariates. Moreover, BMI, trunk fat and total fat were negatively associated with cartilage thickness, and the significant associations disappeared after further adjustment for leptin. Longitudinally, both baseline leptin and change in leptin were associated with greater changes in medial tibial cartilage thickness (β; -0.004, 95% CI: -0.007, -0.001 and β: -0.009, 95% CI: -0.018, -0.001, respectively) in multivariable analyses. In conclusion, serum levels of leptin are independently and consistently associated with cartilage thickness cross-sectionally and longitudinally. In addition, the associations between adiposity measures and cartilage thickness are mediated by leptin suggesting leptin may play a key role in cartilage loss.

Chapter 9 describes the measurement of mean signal intensity of cartilage on T1-weighted MRI images and examines whether mean signal intensity is associated with risk factors and measures of osteoarthritis in younger and older adults. A total of 50 younger adult subjects (mean age 41, range 29-57; 64% female; baseline only) and 168 older adult subjects (mean age 63, range 52-78; 46% female; baseline and 2.9 year followup) were randomly selected from the community. T1-weighted fat-supressed gradient recall echo MRI scans of right knees were performed. Image segmentation was performed semi-automatically, and measures of mean signal intensity and cartilage thickness for regions of

cartilage were obtained. Urinary levels of C-terminal crosslinking telopeptide of type II collagen (U-CTX-II) were measured in younger adults. Cartilage defects were scored using a 5-point scale in both groups. In multivariable analyses, higher cartilage defects and BMI were significantly associated with lower same-region mean signal intensity in younger and older adults. CTX-II was negatively and significantly associated with mean signal intensity of cartilage in the lateral femoral and patellar sites. Joint space narrowing and osteophytes analysed in older adults were significantly associated with reduced mean signal intensity at various sites. Over 2.9 years, lower mean signal intensity at femoral and patellar sites and in whole knee was associated with decreases in cartilage thickness. In conclusion, reduced mean signal intensity of cartilage on T1-weighted gradient recall echo MRI is associated with OA risk factors and predicts cartilage loss suggesting low cartilage signal intensity may reflect early osteoarthritic changes.

Chapter 10 summarises the findings of the thesis and also provides a number of potential directions for future research based on these conclusions.

Chapter 1 - Introduction

1.1 Definition of osteoarthritis

Osteoarthritis (OA) is a chronic disease of the articular joints. It is the most common form of arthritis, and is typified by pain and declining physical function. Knees and hips are the most commonly affected joints. Physiologically, OA is characterised by reduced integrity and metabolic homeostasis of articular cartilage; however, it is now understood that many surrounding tissues are pathologically involved. OA is considered a multifactorial disease, with multiple causal pathways being involved in its aetiology, and has a variety of co- and sub-pathologies in and around the affected joint. Primary OA is an idiopathic disease which may afflict one or two joints or manifest as over a large number of joints in what is known as generalised OA. Secondary OA is attributable to a cause, such as injury or congenital disorders of joints, but also involves many of the same disease processes as primary OA.

Figure 1.1 shows the major anatomical features of the knee and hip. In the knee, the cartilage-covered surfaces of the femoral condyles articulate over and convey load to the similarly covered tibial plateau. The meniscus surrounds the contact area, assisting with dynamic loading and joint stabilisation. The patella is held in place by ligaments and articulates in the trochlear groove, both stabilising and protecting the joint. In the hip, the femoral head forms a ball-joint within the acetabulum. All of the sites with articulating cartilage are susceptible to OA.

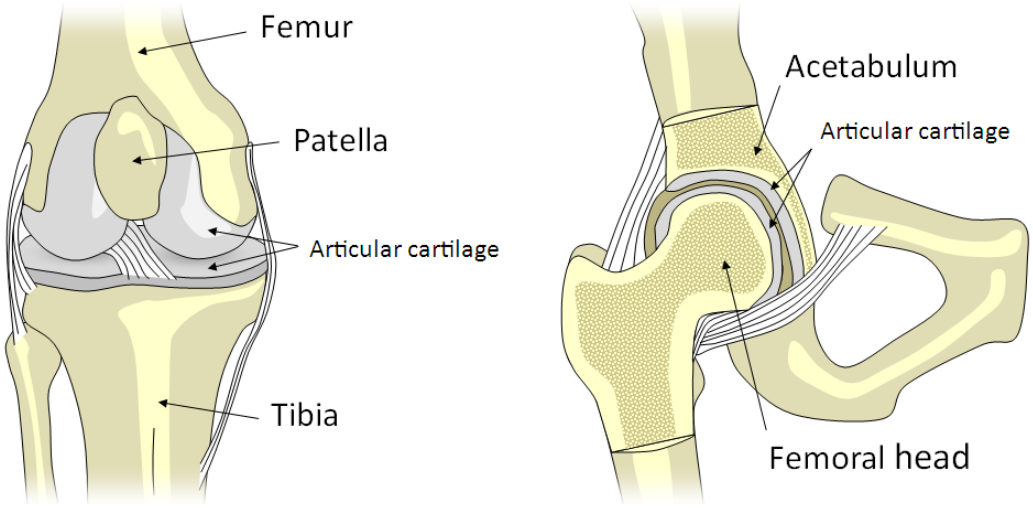

Figure 1.1. Major anatomical features of knee and hip.

OA is a whole-joint disease, affecting a range of tissues including articular cartilage, synovium, subchondral bone, ligaments and periarticular muscle [1]. In a healthy joint, cartilage acts to dissipate the dynamic biomechanical forces through to the surrounding subchondral bone; however, these tissues are susceptible to damage from abnormal external mechanical stress and internal biochemical and morphological changes. Muscles, ligaments and menisci are required to direct and stabilise these forces within the joint, but can fail due to injury or weakness, causing a breakdown in biomechanical function and amplification of physical stresses. The synovium provides a pliable membrane to contain the opposing surfaces of the joint and the synovial fluid within which they operate; however, the synovium in OA can exhibit inflammatory responses which further damage surrounding tissues through the release of pro-inflammatory proteins into the synovial fluid. This interdependence among tissues underpins much of the multifactorial nature of the disease, with loss of healthy function in one tissue directly affecting function in another. This means that OA rarely has a single cause and often presents a variety of pathological features and symptoms.

OA development and progression can be classified into four stages, as shown in Figure 1.2. The changes to the healthy joint in the first stage often involve abnormal growth in the subchondral bone and disruption to normal cartilage homeostasis. This is followed by a second stage involving structural changes in the cartilage and surrounding tissues. Many of these changes can be detected by modern magnetic resonance imaging (MRI). The third stage is represented by definite cartilage loss and bone remodelling including outgrowths known as osteophytes, both of which are visible by traditional radiographic means. Lost cartilage fragments and breakdown products build up in the synovial fluid, interfering with mechanical operation of the joint and stimulating a pro-inflammatory response from the synovium and other nearby tissues. The fourth or final stage is joint death, which is characterised by catastrophic damage to the articulating surfaces and a loss of joint function which can only be treated by joint replacement.

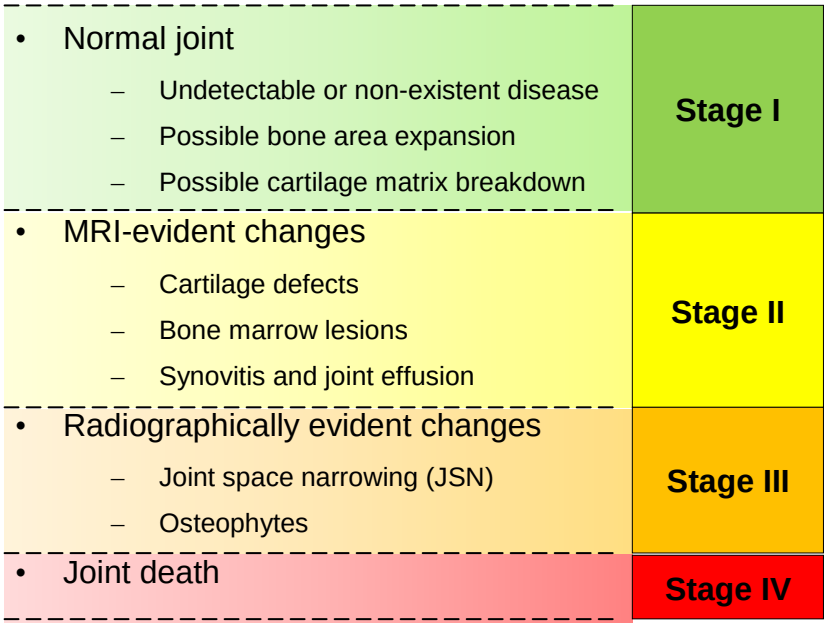

Figure 1.2. Stages of osteoarthritis

1.1.1 Prevalence / burden

OA primarily afflicts older adults; the prevalence of radiographically defined OA in this group is around 27% for both knee and hip OA, and around 7% and 9% respectively for symptomatic knee and hip OA [2]. Approximately 20-25% of older adult Australians have OA of any type, with OA being the largest contributor to the burden of arthritic diseases, which collectively cost the Australian economy $23.9 billion annually [3]. Those who report having OA diagnosed radiographically or otherwise by a medical doctor have consistently more pain, disability and poor health status, and use primary care services more frequently than those who do not [4]. Symptomatic OA in older adults is followed by declining function [5] and lower general health [6]. The prevalence and burden of OA is expected to rise in with increasing life expectancy and an ageing population [7].

1.1.2 Risk factors

OA research over the last few decades has identified several key risk factors for the disease. Systemic risk factors predispose people to OA at multiple joints. The primary systemic risk factor for OA is age, with prevalence of radiographically-defined OA rising from almost zero in young people to over 20% in some groups of older adults [8, 9]. Obesity is a major modifiable risk factor for OA [10]. Obesity is also a moderate risk factor for hip OA with odds ratios between 1 and 3 for incidence [11], and is considered a strong predictor of OA incidence in the knee and hand with respective odds ratios of 2.81 and 2.59 for these sites, although some of the evidence for obesity in hand OA is contradictory [12]. The link with hand OA is considered especially intriguing as it is suggestive of a systemic non-mechanical effect of fat on OA through an altered metabolic and hormonal profiles [13, 14].

Being female has been shown to be a systemic risk factor for higher incidence of OA in both the knee and hand but not hip [15], with being male having an odds ratio of incidence at these sites of 0.63 and 0.81, respectively. This may be due to hormonal or other biochemical differences between the sexes, but might also be due to biomechanical differences in joint size and structure [16, 17]. Hormonal variation has also been implicated in OA. Among females, the use of oestrogen replacement therapy may have a protective effect against OA [18], though the relationship is not clearly defined or established [19]. A variety of studies have shown heritable factors to be involved in OA structural changes [20-22]. Large studies are being undertaken to elucidate the genes involved in OA [23]; some of these studies have uncovered some evidence for variants of genes encoding structural and inflammatory proteins predisposing subjects to OA, though these results will require replication and further investigation [24].

Local risk factors for OA affect joints independently. While some local risk factors may be shared by some joints, local risk factors do not increase the odds of OA in all sites of the body. Outside metabolic effects, obesity can also lead to OA through increases to mechanical loading [25], particularly in the knee [26]. Injury can result in damage to the cartilage, as well as other tissues including ligaments and meniscus, and is a consistent risk factor for the incidence of both knee and hip OA [27, 28]. Injury is understood to predispose subjects to OA through disruption to normal biomechanical function and induction of inflammatory mediators, both of which may negatively affect cartilage metabolism [29].

Malalignment of the knee can increase the odds of OA progression by 4 to 5 times for tibiofemoral OA [30] or 2 times for patellofemoral OA [31]. How the joint is used in everyday activity is important in OA, with occupational activities contributing to OA in all sites [32, 33]. Handedness is considered a possible risk factor, though the evidence is equivocal [34, 35]. Exercise is considered to have dual roles in OA. Mild to moderate non-painful exercise in the absence of existing joint disorder is considered to have a beneficial effect on symptoms [36], and is known to lower the risk of knee OA requiring arthroplasty [37]. There is some evidence to suggest that this may be mediated by muscle strengthening [38]. Higher-intensity exercise and long-term weight-bearing sports are associated with poorer OA outcomes [39, 40]. The more beneficial nature of exercise in unaffected joints underscores the importance of prevention and early intervention in OA.

1.1.3 Clinical Impact

OA is a slowly manifesting disease, with timescales of change measured in many years. Those diagnosed with knee radiographic OA face a slow deterioration of function and increase in knee pain [41]. Disability due to OA can have a profound impact on the quality of life of sufferers. This impact is measured through disability-adjusted life years (DALYs) on a scale of 0 being perfect health to 1 being death, the complement of quality-adjusted life years (QALYs). The currently accepted figures for Australia give mild, moderate and severe OA as having respective DALY factors of 0.01, 0.21 and 0.48 [3]. OA is a major burden on the healthcare system, with costs for treatment of arthritic conditions in Australia is estimated at 4.8 billion dollars annually [3].

Pain is the major symptom of OA. Prevalent radiographic OA of the knee and individual radiographic features are associated with knee pain [42]; however, radiographic changes can occur without any occurrence of symptomatic pain, and vice versa [43]. This may be due to the fact that cartilage tissue lacks pain fibres necessary for pain sensation and that pain may originate from a variety of tissues in and around the joint. Bone is a dynamic, innervated tissue, and changes in subchondral bone such as bone marrow lesions and other remodelling may be responsible for some reported pain [44-46]. However, the extent to which bone contributes to joint pain is not clear. There is also evidence to suggest that some pain in OA may be derived from inflammation in synovial tissue [47] and, in the knee, possibly from damage to the meniscus [48].

There is some evidence that those with symptomatic OA can suffer some degree of central pain sensitisation [49], which is characterised by the persistent abnormal sensitivity to either nociceptive input (hyperalgesia) or innocuous stimuli such as soft touch or mild temperature variation (allodynia) [50]. Research in murine models supports this hypothesis, and suggests that this may involve inflammatory factors [51-53].

The later stages of OA can involve impaired function. This is mainly due to pain [5]. However, the course of functional deterioration in the hip and knee is slow; clear loss of function is not seen at a group level in less than three years [41].

OA is a common indication for both total knee replacement (TKR) and total hip replacement (THR), which are typically performed in joints by near complete loss of cartilage and severe pain or loss of function. TKR and THR are the only therapeutic interventions applicable to end-stage disease.

1.1.4 Treatment

Despite the high prevalence and severe economic impacts of OA, treatment and prevention options are quite limited. OA is a largely non-remitting disease, and interventions combatting OA are typically focussed on prevention of incidence and stopping progression.

In earlier stages of OA, the disease favours lifestyle interventions, particularly exercise to increase muscle strength. In the later stages, treatment relies on palliative therapy and reducing the risk of further damage through improper biomechanical loading. End-stage OA, which is characterised by complete loss of cartilage and severe impediment to function, is treatable only by joint replacement.

The treatment of OA continues to evolve as basic and clinical science change treatment practices. The American Academy of Orthopaedic Surgeons curate guidelines for clinical practice in treating OA [54].The first line of treatment is the prescription of muscle strengthening and neuromuscular training [54]; moderate exercise is known to have protective effects in OA in the absence of injury [36]. In symptomatic OA patients classified as overweight by having a BMI of 25 or over, weight loss is recommended [54]. Weight loss improves pain and function and helps delay structural progression [55].

While there are no approved disease-modifying anti-osteoarthritis drugs (DMOADs) for use in humans, symptoms can be treated pharmacologically. As a result, pharmacological treatment of OA focuses on the relief of symptoms through analgesics such as acetaminophen [56, 57], or the more efficacious non-steroidal anti-inflammatory drugs (NSAIDs) [56], which can induce side-effects and may hasten cartilage loss [58, 59]. Dietary supplements containing cartilage synthesis precursors derived from animal tissue, such as glucosamine and chondroitin, are becoming popular self-administered treatments for OA. However, a recent systematic review suggests they may not have any effect [60] and they are not generally recommended in clinical practice [54].

The testing of new drugs relies on sensitive and accurate outcome measures in clinical trials designed to test the efficacy and safety of candidate drugs. As OA development and progression can take place over many years, it is necessary to be able to select samples of subjects for randomisation who are at high risk of incidence or progression. The range of joints and compartments afflicted, as well as the range of structural changes and symptoms experienced in OA are acknowledged as a limitation when trying to generate a single definition of the disease [61], and this complexity and

multifactorial nature of OA makes it difficult to predict disease development. The development of new biochemical and imaging measures may help to better define those at risk of incidence and progression [62]

Surgical interventions are generally only applied when treatment by other means is no longer effective [56]. Arthroscopically performed lavage and debridement of an affected joint to smooth rough portions of cartilage or meniscus and removes loose fragments of tissue are recommended in practice [54]. Osteotomy is a surgical procedure involving the removal of bone to reshape joints, often to correct malalignment. Osteotomy is used in practice [63], but may be obviated by joint replacement. Joint replacement is the removal of large portions of bone and cartilage and substitution with artificial articulating surfaces, either for one or both opposing surfaces. Joint replacement improves both symptoms and function in OA and is becoming more common in practice [64].

Other less commonly used surgical techniques aimed at conserving joint health and encouraging repair are bone marrow stimulation and autologous chondrocyte implantation. Autologous chondrocyte implantation is performed to encourage cartilage repair in damaged regions by transplanting cultured chondrocytes harvested from less weight-bearing cartilage, while bone marrow stimulation draws pluripotent stem cells from the subchondral bone and uses them to stimulate cartilage repair [65].”

1.1.5 Biomarkers in osteoarthritis

A biomarker is a measure of a subject which can be used to describe, classify or predict the state of a disease. The term has evolved from applying only to biochemical measures and now has a broader definition which covers any measure of disease, including those derived from imaging methods.

Biomarkers are crucial to both the accurate diagnosis and for clinical treatment of OA, as well as to the research effort to investigate new targets for therapeutic intervention.

The Osteoarthritis Biomarkers network (funded by the US National Institute of Health), has provided a classification scheme for OA biomarkers comprising five categories: Burden of disease; Investigative; Prognostic; Efficacy of intervention; and Diagnostic biomarkers [66]. These categories are described below.

Diagnostic

A diagnostic biomarker is assessed for power to classify subjects into those with present or absent disease. A Kellgren–Lawrence (KL) grade of radiographic OA of 2 or

more is the most widely accepted diagnostic marker in OA. Diagnostic markers can be used in clinical practice to classify patients or in research to measure incidence in cohort groups.

Prognostic

A prognostic biomarker is one which is able to predict either incidence or progression of OA severity. Incidence and progression are often defined as a change in diagnostic state, e.g. attaining a KL grade of 2 or increasing by a KL grade unit.

Burden of Disease

A disease burden biomarker differentiates between different levels of OA. These biomarkers are useful in determining the disease activity in an individual. Examples of current burden markers include markers of cartilage breakdown such as cartilage oligmeric matrix protein (COMP) and C-terminal crosslinking telopeptide of type II collagen (CTX-II). Clinical applications include the monitoring the disease status of a patient over time. Burden markers can be used in research as fine measures of OA for detecting differences in disease severity between study groups.

Efficacy of Intervention

A biomarker measuring efficacy of intervention must be able to be used as a surrogate for positive response to treatment. Changes in efficacy biomarkers must also predict better outcomes for subjects. Any measure which does as such can be used to establish the effect of a treatment over controls in clinical trials.

Investigative

An investigative marker is a measure of a biological state or process, which does not provide definitive information about the current or future clinical state of the subject. Investigative markers are commonly used in preliminary research to measure biological processes which are suspected to contribute to disease, where they can help uncover new diagnostic and targets for treatment.

This thesis examines a number of biochemical and imaging biomarkers. The utility and potential pathological processes implicated by these biomarkers are not established within the OA research field at present; the rationale for their consideration for further study is outlined in the following sections. As such, the majority of biomarkers examined here fall under the ‘Investigative’ descriptor. Where successful validation of a biomarker would lead to categorisation as a ‘Burden’ or ‘Prognostic’ indicator, this is highlighted.

1.2 Inflammation and obesity in osteoarthritis

Inflammation is a common feature in OA, principally involves the synovial tissue. Synovitis can occur before any radiographically evident changes [67] and is believed to play a role in both OA symptoms and cartilage damage [68].
Inflammation of the synovium may also contribute to joint pain [69, 70].
However, inflammatory factors may also originate outside the affected joint. Inflammatory cytokines such as IL-1β, TNF-α and IL-6 have been associated with the aetiology of OA [71].

Obesity is also linked to systemic inflammation. Obesity is often accompanied by a combination of low-grade inflammation [72] and metabolic dysfunction [73]. This metabolically triggered inflammation is largely driven by adipokines, which have a role in regulating energy intake and metabolism. Of the adipokines, leptin is the most studied. Leptin is a protein produced primarily by adipocytes in fat tissue and is classified as an adipocytokine [74], being able to up-regulate inflammatory factors systemically through influence on a variety of tissues [75]. Differences in body morphometry are observed to be directly associated with levels of leptin measured within the joint [76]. As well as stimulating synthesis of pro-inflammatory mediators such as nitric oxide (NO) [77], leptin may also have a direct effect on cartilage.

It is unclear whether metabolic and inflammatory mediators are associated with or predict OA structural changes and symptoms. If the associations exist, the findings would potentially support new treatments and allow for these biomarkers to be used as prognostic biomarkers in OA. These topics are investigated in Chapter 4, Chapter 5, Chapter 6 and Chapter 8.

1.3 Measurement methods and diagnostic criteria

As a disease of multiple stages and pathways afflicting a variety of tissues in a various joints, various measures are currently being used or developed for clinical diagnosis and research quantification of OA.

1.3.1 Radiographic

Due to the relative ease of radiographic imaging, and the existence of long-standing x-ray diagnostic criteria, radiographic measures are the currently accepted standard for diagnosis of OA in research studies. The major feature of radiographic OA is joint space narrowing (JSN), which is a surrogate measure for cartilage loss. However, JSN is an imperfect measure, and can be affected by variations in imaging parameters, joint positioning, reader bias and, in the knee, meniscal extrusion.

Osteophytes are bony outgrowths of the bones of the knee, hip and hand which are considered features of OA. Osteophytes are considered a hypertrophic response of subchondral bone tissue, at the margins of the joint, in response to changes in joint loading [78]. It is not clear whether osteophytes are contributors to further degradation; furthermore they may reflect a protective response of the bone against joint instability [79]. The relationship of osteophytes as an independent causal factor in either OA structural or symptomatic progression is controversial. Sclerosis or hardening of the bone is a further effect of the bone remodelling that occurs in OA and is visible on conventional radiographs [80, 81].

The radiographs shown in Figure 1.3 depict the appearance of joint space narrowing and osteophytes in affected knees and hips relative to unaffected joints.

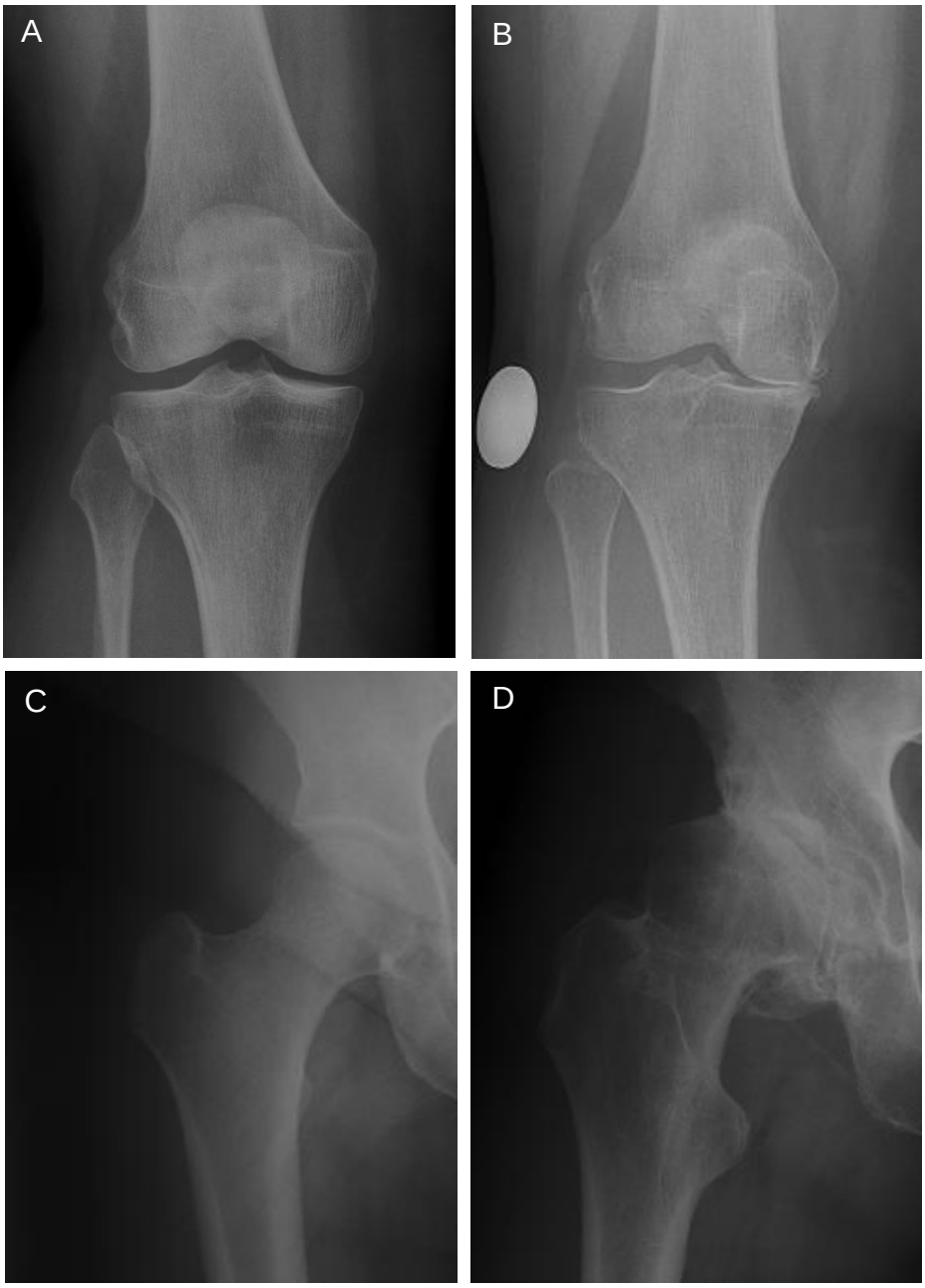

Figure 1.3. X-ray images of knees and hips unaffected and affected by osteoarthritis. A. Right knee with no radiographic osteoarthritis. B. Right knee showing severe medial joint space narrowing and moderate medial femoral and medial tibial osteophytes. C. Right hip with no radiographic osteoarthritis. D. Right hip with severe superior and axial joint space narrowing and severe femoral and acetabular osteophytes.

The accepted radiographic gold standard for diagnosis of OA is the Kellgren–Lawrence (KL) grade [82], shown in Table 1.1, which reflects both the severity of joint space narrowing and growth of osteophytes within a joint on a single axis of progression. Prevalent OA is commonly defined in OA research by a cutoff KL grade of two or more.

Table 1.1. Kellgren–Lawrence osteoarthritis grade

Kellgren–Lawrence Grade of Osteoarthritis	**Description**
0 None	No osteoarthritis.
1 Doubtful	Doubtful narrowing of joint space and possible osteophytic lipping.
2 Mild	Definite osteophytes and possible narrowing of joint space.
3 Moderate	Multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends.
4 Severe	Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends.

More recently developed scales, such as the Osteoarthritis Society International (OARSI) radiographic atlas [83, 84], define each discernible radiographic characteristic of OA separately, in the hand, hip and knee. This scoring system has the advantage of giving each radiographic feature a distinct score, whereas a composite measure such as the KL grade may hinder the understanding of the separate components of radiographic OA. The features encompassed in the OARSI atlas are described in Table 1.2.

Table 1.2. Osteoarthritis Research Society International atlas of radiographic features (knee and hip)

Site and feature	Description			
Knee (tibiofemoral)				
Marginal osteophytes				
Medial femoral condyle	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Medial tibial plateau	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Lateral femoral condyle	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Lateral tibial plateau	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Joint space narrowing				
Medial compartment	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Lateral compartment	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Other				
Medial tibial attrition	0 (absent)		1 (present)	
Medial tibial sclerosis	0 (absent)		1 (present)	
Lateral femoral sclerosis	0 (absent)		1 (present)	
Hip				
Marginal osteophytes				
Superior acetabular	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Superior femoral	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Inferior femoral	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Inferior acetabular	0 (absent)		1 (present)	
Joint space narrowing				
Superior	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Medial	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Other				
Acetabular subchondral cyst	0 (absent)		1 (present)	
Femoral subchondral cyst	0 (absent)		1 (present)	
Flattening of femoral head	0 (absent)		1 (present)	
Thickening of medial femoral calcar	0 (absent)		1 (present)	

1.3.2 Magnetic resonance imaging

MRI is an increasingly popular tool for evaluating the presence and severity of OA. The main advantage of MRI over traditional radiographic means is the ability to detect early OA changes not discernible on x-ray. As such, MRI plays an important role in the early diagnosis of OA and assessing its severity [85].

Loss of cartilage volume is the primary measure of knee OA progression to be measured from MRI. Cartilage volume measurement is considered an accurate method for assessing cartilage loss and changes in cartilage volume predict future need for surgical intervention [86]. Due to natural variation in bone size and joint morphology, cartilage volume does not have the power to specifically and sensitively classify OA in individuals.

While many groups move towards adopting cartilage volume as a standard measure of OA progression, recent focus has been given to the use of cartilage thickness as an alternative measure. Cartilage thickness has the advantage of being able to measure cartilage independent of inter-subject differences or intra-subject changes in bone size. Cartilage thickness is negatively associated with radiographic joint space narrowing in the knee [87].

Bone marrow lesions (BMLs), known previously as bone marrow edema (BME), are regions of abnormal tissue within the subchondral bone, seen as darker patches within the bone on T1-weighted MRI and lighter patches on T2-weighted MRI. These imaging features represent an increase in water and unsaturated fats within the lesion [88] and often involve pathological changes such as bone marrow necrosis and fibrosis, as well as abnormal trabecular structure [89]. BMLs will appear and disappear in older adults [44]. The invisibility of BMLs in conventional radiographic imaging, coupled with their location within the bone, has left them relatively unstudied and not well understood. MRI has made it apparent that BMLs are far from static; subjects with established OA experience BMLs which can appear or disappear and often fluctuate in size[90]. BML changes and changes in pain appear to be linked only in unaffected and early OA subjects [44, 90]. Knee BMLs predict development of cartilage defects and loss of cartilage volume on a same-site basis[91], and also predict increased incidence of total knee replacement (TKR) [44].

Cartilage defects are localised lesions or tears within the cartilage, visible on T1- or T2-weighted MRI, and are a common element of knee OA [92]. Considered a pre-radiographic OA feature, knee cartilage defects are known to be common in younger and middle-aged adults, where they often regress [93, 94]. Cartilage defects may be due in part

to genetic factors and share a common causal pathway with subchondral bone remodelling [21]. Knee cartilage defects in younger adults are associated with radiographic OA and loss of cartilage volume over time [95, 96]. Their use as a burden-of-disease and prognostic marker of OA features and outcomes in older adults is less examined; Chapter 7 focusses on this question.

The use of MRI to visualise and assess many different disease features of OA has encouraged research groups to try to develop holistic measures of joint health and OA progression which generate overall severity scores. These mostly function by summing semi-quantitative scales of a range of disease features in various tissues and compartments. Examples for the knee include the Knee Osteoarthritis Scoring System (KOSS) (83), the Whole Organ Magnetic Resonance Imaging Score (WORMS) (84), the Boston Leeds Osteoarthritis Knee Score (BLOKS) (85) and the MRI Osteoarthritis Knee Score (MOAKS). Similar scoring systems for other joints include the Hip Osteoarthritis MRI Scoring System (HOAMS) [97] and the Oslo Hand Osteoarthritis MRI score (OHA-MRI) [98].

A recent trend of OA research has been to directly examine the state of cartilage using quantitative MRI. Various methodologies have been developed which aim to reflect differences in cartilage health in pre-radiographic OA populations. The most popular ones include measures of relaxation time from T2 and T1ρ sequences, which examine early degenerative changes [99-102], as well as Delayed Gadolinium-enhanced MRI of cartilage (dGEMRIC) [103, 104], which makes use of a contrast medium to examine loss of proteoglycan within the cartilage matrix. These methodologies require expensive or specialty sequences.

There is some evidence that differences in signal intensity of cartilage observed on conventional T1 MRI may reflect the very early stages of cartilage damage structure [105]. This measure is examined as an investigative biomarker in Chapter 9.

Chapter 2 - Research Questions

In population-based samples of community-dwelling adults aged 26-61 and 50–80 years respectively, examined at baseline for both groups and for the older adults again approximately 3 and 5 years later:

1) What are the cross-sectional associations of both leptin and IL-6 with hip OA severity in older adults?

2) What are the associations of both IL-6 and TNF-α with knee radiographic OA and cartilage loss in older adults?

3) What are the associations in older adults of both baseline and early changes in IL-6, TNF- α and high-sensitivity C-reactive protein (hs-CRP) and changes in knee pain over a longer time period?

4) What is the natural history of knee cartilage defects in older adults, which factors are associated with cartilage defects and do cartilage defects predict future cartilage loss and joint replacement?

5) Are serum levels of leptin associated cross-sectionally with quantity of knee cartilage as measured by mean cartilage thickness in older adults, and do levels of leptin predict changes in cartilage thickness?

6) What are the associations between mean signal intensity of knee cartilage on T1-weighted MRI and OA risk factors in younger and older adults, and does T1 mean signal of cartilage predict changes in knee cartilage thickness?

Chapter 3 - Methodology

3.1 Prelude

This thesis arose from analyses conducted primarily in older adults, using data from the Tasmanian Older Adult Cohort (TASOAC). Analysis in Chapter 10 was augmented by a sample of 50 subjects from the Knee Cartilage Volume Study (KCVS) study. Both studies are similar in some aspects of methodology, particularly in the outcome factors, study factors and covariates which have been measured.

This chapter describes each study population and the measurement protocols they use, which are referred to in subsequent chapters. The following chapters are presented in the form in which they were submitted to or published by respective journals. Thus, the papers may vary in their description of methods, analyses, results and interpretations.

3.2 TASOAC study population and design

The TASOAC study is a prospective, population-based cohort study aimed at identifying the environmental, genetic, and biochemical factors associated with the development and progression of OA of the hand, knee, hip, and spine. The study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee, and written informed consent was obtained from all participants.
The first phase of the study was carried out in southern Tasmania from March until August 2002. The followup study was conducted 2.7 years later (range 2.6-3.3 years), with a set of measures taken also at a second follow-up after 5 years (range 5.3 – 6.8 years; average 5.6 years). The study group consisted of older adults between the ages of 50 and 80 (mean: 62 years; standard deviation (SD): 7 years), who were randomly selected from the electoral roll of Southern Tasmania (population 229,000), which is used for compulsory elections and carries the most complete data on the local population. The scope of the study was limited to community-dwelling adults and institutionalised persons were excluded. Participants were also excluded if they reported any contraindications for MRI procedures required for the study (including metal sutures, presence of shrapnel, iron filings in the eye and claustrophobia). The recruitment process for the study is described in Figure 3.1.

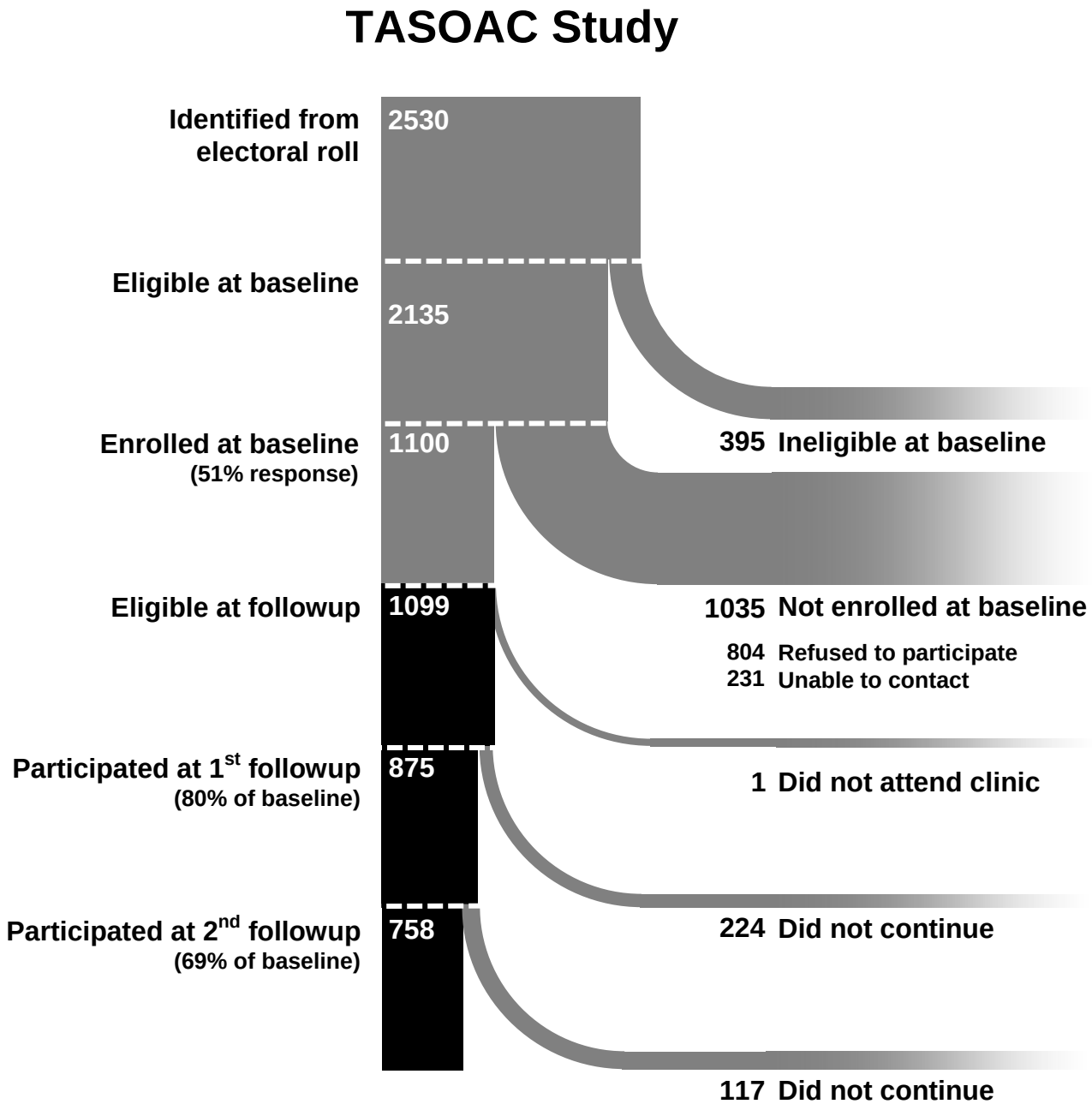

Figure 3.1. Flowchart of TASOAC study participation.

The measures taken in this study and their accompanying methodologies are discussed in detail below.

3.2.1 Subject characteristics

Age, sex and the presence of diseases, including heart disease, diabetes, asthma and rheumatoid arthritis, were recorded by questionnaire at baseline.

3.2.2 Physical activity

Physical activity as represented by steps per day was measured using a pedometer worn on their dominant side for seven consecutive days except during sleeping or water based activities.

3.2.3 Anthropometrics

Height was measured to the nearest 0.1 cm (with shoes, socks, and headgear removed) using a stadiometer. Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707, Bradford, MA) that were calibrated using a known weight at the beginning of each clinic. BMI was also calculated:

$$\text{BMI} = \frac{\text{weight (kg)}}{\text{height (m)}^2}$$

Waist and hip circumference were measured and waist-to-hip ratio (WHR) was calculated:

$$\text{WHR} = \frac{\text{waist circumference}}{\text{hip circumference}}$$

Total body and trunk fat mass (kg) was measured by a Hologic dual energy x ray absorptiometry (DXA) scanner (Hologic Corp., Waltham, Massachusetts, USA).

3.2.4 Serum biomarker measurements

In a sub-sample of subjects for both baseline and first followup, serum was isolated and refrigerated overnight in plastic tubes, at which time aliquots were prepared and stored at -80°C. IL-1 β, IL-6 and TNF-α were measured at baseline and then at first followup with

a solid-phase, two-site chemiluminescent enzyme immunometric assay method by use of IMMULITE IL-1 β, IMMULITE IL-6 and IMMULITE TNF-α (all from EURO/DPC Llanberis, Gwynedd, United Kingdom). Samples with undetectable cytokine concentrations were assigned a value corresponding to the lower limit of detection of the assay (1.5 pg/ml for IL-1 β, 2 pg/mL for IL-6 and 1.7 pg/mL for TNF-α). The coefficients of variation (CVs) in our hands were 3% for IL-1 β, 8% for IL-6 and 6% for TNF-α [106].

Testing high-sensitivity CRP (hs-CRP) was performed by using the CRP-Latex (II) immunoturbidimetric assay (Abbott Diagnostic's c8000 Architect). The lower detection limit of the assay is 0.01 mg/L. The CV in our hands was of the order of 4.8% [106].

3.2.5 Radiographic measurements

A standing anteroposterior semiflexed view of the right and left knees with 15^0 of fixed knee flexion was performed in all subjects at baseline and scored individually for osteophytes and joint space narrowing (JSN) on a scale of 0-3 (0 = normal and 3 = severe) according to the Osteoarthritis Research Society International (OARSI) atlas as previously described [107].

Anteroposterior radiographs of the pelvis with weight bearing and with both feet in 10° of internal rotation were obtained. Radiographic features of joint space narrowing (JSN) (superior and axial) and osteophytes (superior femoral and superior acetabular) of the left and right hip were graded on a 4-point scale (range 0–3, where 0 = no disease and 3 = most severe disease) using the Altman atlas [84] as previously described [108]. Each score was arrived at by consensus between 2 readers who simultaneously assessed the radiograph, with immediate reference to the atlas. The intraobserver reliability was assessed in 40 subjects with intraclass correlation coefficients of 0.60–0.87 [108].

3.2.6 Joint pain assessment

Knee pain (on flat surface, going up/down stairs, at night, sitting/lying and standing upright) was assessed at baseline and 5-year followup by self-administered questionnaire using the Western Ontario McMaster Osteoarthritis Index (WOMAC) with a 10-point scale from 0 (no pain, stiffness or no function problems) to 9 (most severe pain, stiffness or severe function problems) [109]. These scores were summed to create a total score from 0 to 45.

Hip pain was assessed by questionnaire at baseline and was defined as pain for >24 hours in the last 12 months or daily pain on more than 30 days of the last year.

3.2.7 Knee replacement surgery

At the 5 year follow-up participants were asked whether they had undergone a total knee replacement since their first visit. Although MRI scans were taken of the right knee only, replacement surgery data were collected for both knees.

3.2.8 MRI acquisition

MRI scans of the right knee were performed at baseline and first follow-up. Knees were imaged in the sagittal plane on a 1.5-T whole body magnetic resonance unit (Picker, Cleveland, OH) equipped with a commercial transmit–receive extremity coil and a fat-saturated T1-weighted spoiled gradient echo and T2-weighted fast spin echo sequences were used.

The following two image sequences were used for both baseline and followup measures: T1-weighted fat saturation 3D gradient recall acquisition in the steady state, flip angle 30°; repetition time 3067ms; echo time 112msec; field of view 16 cm; 512 x 512–pixel matrix with an in-plane resolution of 0.31 x 0.31 mm; ~60 slices with 1.5mm thickness without inter-slice gap; acquisition time 5 minutes 58 seconds, 1 acquisition; T2-weighted fat saturation two-dimensional (2D) fast spin echo, flip angle 90°; repetition time 31 ms; echo time 6.71 msec; field of view 16 cm; 256 x 256–pixel matrix with an in-plane resolution of 0.63 x 0.63 mm; ~15 slices with 4mm thickness with an interslice gap of 0.5–1.0 mm.

3.2.9 Knee cartilage volume

Knee cartilage volume was determined unpaired and unblinded to sequence on baseline and followup images by means of image processing on an independent workstation as previously described [110, 111]. The volumes of individual cartilage plates (medial tibial, and lateral tibial) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis. These data were then resampled by means of bilinear and cubic interpolation (area of 312

and 312 μm and 1.5 mm thickness, continuous sections) for the final 3D rendering. The CVs for cartilage volume measures in our hands were 2.1-2.6% [110].

3.2.10 Knee cartilage defects

Defects were graded from T1 MRI at baseline and first followup unpaired and unblinded to sequence by a trained observer at the medial tibial, medial femoral, lateral tibial, lateral femoral and patellar sites as follows [93, 96, 112]: grade 0 = normal cartilage; grade 1 = focal blistering and intracartilaginous low-signal intensity area with an intact surface and bottom; grade 2 = irregularities on the surface or bottom and loss of thickness of less than 50%; grade 3 = deep ulceration with loss of thickness of more than 50%; grade 4 = full-thickness chondral wear with exposure of subchondral bone. A cartilage defect also had to be present in at least two consecutive sections. If multiple defects existed at one site, the highest grade was used. The reader was unaware of the initial result at the time of the second reading. Intraobserver reliability (expressed as intraclass correlation coefficient) was 0.89 to 0.94 [96].

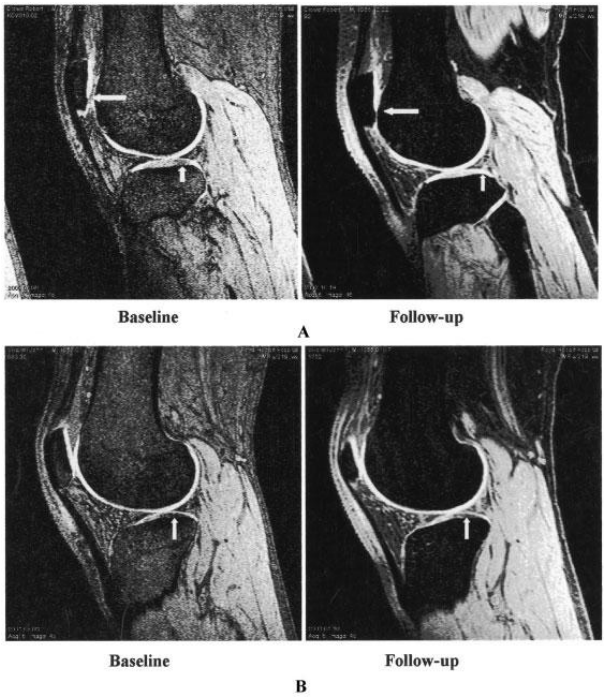

Figure 3.2. Change in knee cartilage defects grades over 2 years in 2 different subjects. A, In this subject, the patellar cartilage defect (long arrow) is grade 2 at baseline and grade 4 at followup. The tibial cartilage defect (short arrow) is grade 1 at baseline and grade 0 at followup. B, In this subject, the tibial cartilage defect (arrow) is grade 3 at baseline and grade 1 at followup.

3.2.11 Bone marrow lesions

Bone marrow lesions were assessed using fat-suppressed T2-weighted MR images by a trained observer at baseline as previously described [91]. Each BML was scored 0-3 on the basis of lesion size (Grade 1 if it was only present on one slice, Grade 2 if present on two consecutive slices, Grade 3 if present on three or more consecutive slices). The BML with the highest score was used if more than one lesion was present at the same site. The ICCs for MRI measures of BMLs and cartilage defects were 0.80-1.00.

3.2.12 Effusion

Suprapatellar effusion was scored visually, blinded to sequence and without pairing, using the Boston-Leeds Osteoarthritis Knee Score [113] as either present (≥1) or absent on the baseline T2 MRI images, with κ=1.00.

3.2.13 Knee bone size

Knee tibial plateau bone areas were determined from T1 MRI by means of image processing in an independent work station unpaired and unblinded to sequence using the software program Osiris as previously described [114]. The bone area of the medial and lateral tibial plateau is uniform in nature and was directly measured from the reformatted axial images. The CVs for these measures in our experience are 2.2-2.6% [114].

3.2.14 Semi-automated segmentation

Segmentation was performed blinded to sequence and without pairing, in the baseline and followup T1 MRI scans of 168 subjects who had both baseline and followup serum samples taken, using custom semi-automated segmentation software written in MATLAB. The semi-automated approach used the following method. First the user selected start and end sagittal images for each major cartilage region (femoral, medial tibial, lateral tibial and patellar), as well as seed points in the subchondral bone midway between the two slices. Initial boundary finding for the bone–cartilage interface was performed by an active contour approach. The contour was seeded as a thin cylindrical mesh along the sagittal axis and grew outwards, where the user was able to adjust coefficients relating to internal and image forces to find a good fit. After fine adjustment of

this inner contour by grayscale smoothing and thresholding, a 2D grayscale image was presented representing the mean signal intensity over several pixels outward from the subchondral bone–cartilage interface. The user was able to delineate the edges (boundary where inner and outer surfaces meet) of the cartilage region. A second active contour projected outwards, controlled by the user, was used to find the outer surface of the cartilage. The final stage involved checking and manual adjustment of contours in individual slices to correct any errors.

This method allowed for non-contiguous portions of cartilage to be grouped together, and was sensitive to portions of cartilage unconnected in the same slice (Figure 3.5A). For our analysis, femoral cartilage was considered as a single region, as was patellar cartilage. Medial and lateral tibial portions of cartilage were considered as separate whole regions. Analysis was also performed using all knee cartilage combined. The average time to segment each subject was 29 minutes.

3.2.15 Mean cartilage signal intensity on T1 MRI

The mean signal intensity was measured in all semi-automatically segmented T1 MRI scans from the entire sample of voxels within a region of cartilage, measured over all relevant slices, as shown in Figure 3.3B. Signal intensity of each voxel measured from the image is proportional to both the proton density and the shortness of the T1 relaxation time at the specific point of the tissue being examined, both of which vary between and within tissues. Measures were and repeated in a randomly selected sample of TASOAC participants (n=20) to assess reproducibility, with measures for both original and second segmentations not being calculated until after repetition. Intra-observer reproducibility for mean intensity in all cartilage and each region was less than 1.5%.

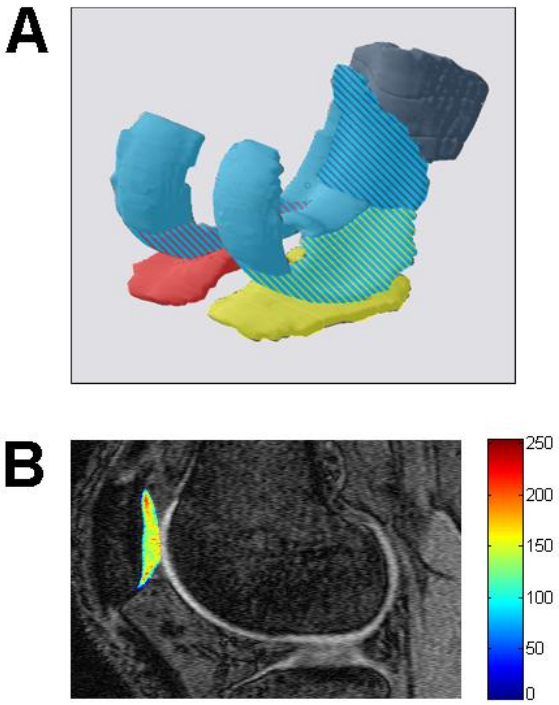

Figure 3.3. A). Cartilage segmentation regions. 1. cyan: femoral; 2. cyan/red: femoral overlying medial tibia; 3. cyan/yellow: femoral overlying lateral tibia; 4. cyan/dark blue: femoral overlying patella; 5. red: medial tibial; 6. yellow: lateral tibial; 7. dark blue: patellar. B). Mean intensity calculation. Each sub-region was segmented as a region of interest (ROI) in each slice in which any of its voxels were contained. Mean intensity for each sub-region was calculated as the average of the voxel-specific values within all ROIs of that sub-region in all slices. A typical ROI for one slice of patellar cartilage is shown in false color.

3.2.16 Mean cartilage thickness

Mean thickness for a region of cartilage was calculated in all semi-automatically segmented T1 MRI scans as the mean distance from inner to outer surface, from a sample of uniformly spaced points over the entire cartilage-covered surface as shown in Figure 3.4. Change in mean thickness was calculated as [followup - baseline] for each region. Intra-observer reproducibility (measured in 20 subjects) for mean cartilage thickness, as measured by coefficient of variation (CV), was 1.9-2.9%.This is similar to that for cartilage volume in our hands [107].

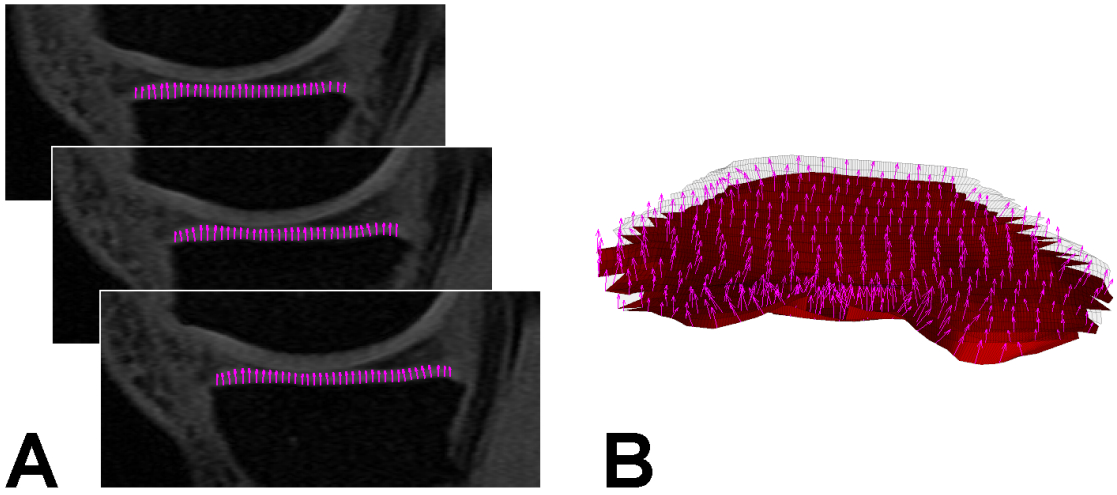

Figure 3.4. Semi-automated cartilage thickness measurement. A) Measurements of thickness at regularly sampled points in typical adjacent slices of medial tibial cartilage. B) Representation of thickness measures over entire medial tibial plate (spacing down-sampled in both images for clarity).

3.3 KCV study population and design

The KCV study was conducted using participants recruited and measured from Southern Tasmania during the period from June 2000 to December 2001. Both males and females (64% female) aged from 26 to 61 (mean: 45 years; SD: 7 years). The larger study was designed to investigate the genetic contributions towards OA and included subjects selected from two sources; firstly the adult children of subjects who had had a knee replacement performed for primary knee OA at any Hobart hospital in the 5 years prior; and secondly a group of control subjects randomly selected from the electoral roll in a manner similar to that in the TASOAC study. The study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee, and written informed consent was obtained from all participants. Participants were excluded for MRI contraindications as in the TASOAC study. No women were on hormone replacement therapy at the time of the study. Subjects with knee pain were allowed. Only a sub-selection of this control group was used for analysis in this thesis; specifically, the first 50 subjects who had baseline MRI and u-CTX-II measures available were selected and had their T1-weighted MRI scans undergo semi-automated image segmentation and analysis. The recruitment process for these subjects is outlined in Figure 3.5.

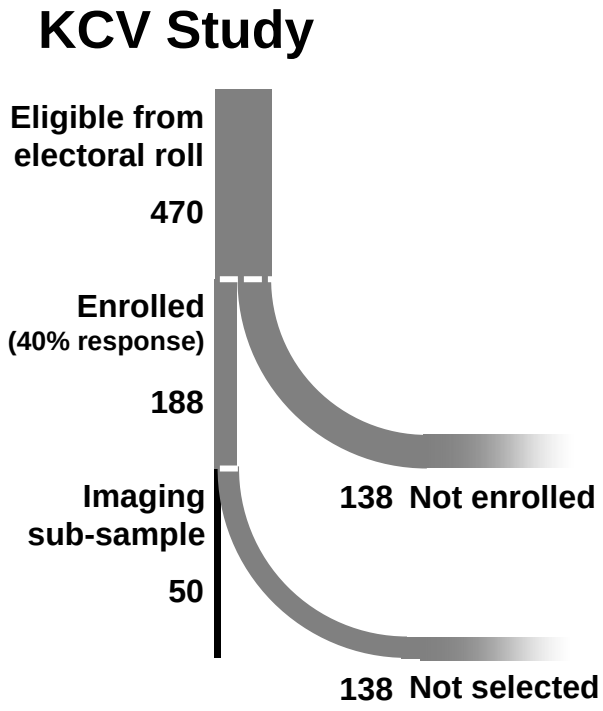

Figure 3.5. Flowchart of KCV study participation.

3.3.1 Subject characteristics

Age and sex were recorded.

3.3.2 Anthropometrics

Height and weight were measured as described in section 3.2.3, and BMI was calculated.

3.3.3 MRI acquisition

T1 weighted MRI scans were taken for each of the 50 subjects using the same protocol as described in section 3.2.8.

3.3.4 Radiographic measures

Knee x-rays were obtained and scored for JSN and osteophytes using the same protocol as described in section 3.2.5.

3.3.5 Urinary CTX-II

Overnight urine samples were collected in plastic containers from the younger adult group. After mixing the whole collection, aliquots of urine were transferred into plastic tubes and frozen at -70°C without any acidification. U-CTX-II was measured by an enzyme-linked immunosorbent assay based on a monoclonal antibody raised against the EKGPDP linear six amino acid epitope of the type II collagen C-telopeptide (Cartilaps, Nordic Bioscience, Herlev, Denmark). Intra- and inter-assay CVs are lower than 8% and 10%. U-CTX-II was corrected for urinary creatinine levels.

3.3.6 Semi-automated segmentation

T1-weighted MRI scans were segmented semi-automatically using the same software approach as described in section 3.2.14.

3.3.7 Mean cartilage signal intensity on T1-weighted MRI

Mean T1 signal intensity of cartilage was measured for each region as described in section 3.2.15.

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Chapter 4

The association between leptin, interleukin-6 and hip radiographic osteoarthritis in older people: A cross-sectional study

Published in:

http://www.ncbi.nlm.nih.gov/pubmed/20482813

Stannus OP, Jones G, Quinn SJ, Cicuttini FM, Dore d, Ding C. 2010. The association between leptin, interleukin-6 and hip radiographic osteoarthritis in older people: A cross-sectional study. *Arthritis Research and Therapy*, 12 (3).

doi: 10.1186/ar3022

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Chapter 5

Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults

Published in:

http://www.ncbi.nlm.nih.gov/pubmed/20816981

Stannus OP, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J, Ding C. 2010. Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. *Osteoarthritis and Cartilage / OARS, Arthritis Research Society, 18 (11).*

doi: 10.1016/j.joca.2010.08.016

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Chapter 6

Associations between serum levels of inflammatory markers and change in knee pain over 5 years in older adults: a prospective cohort study

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Stannus OP, Jones G, Blizzard L, Cicuttini FM, Ding C. 2013. Associations between serum levels of inflammatory markers and change in knee pain over 5 years in older adults: a prospective cohort study. *Annals of the Rheumatic Diseases, 72 (4), pp535-40.*

doi: 10.1136/annrheumdis-2011-201047

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Chapter 7

Knee cartilage defects in a sample of older adults: natural history, clinical significance and factors influencing change over 2.9 years

Published in:

http://www.ncbi.nlm.nih.gov/pubmed/22960091

Carnes J, Stannus O, Cicuttini F, Ding C, Jones G.2012. Knee cartilage defects in a sample of older adults: natural history, clinical significance and factors influencing change over 2.9 years. *Osteoarthritis and Cartilage / OARS, Arthritis Research Society.*20 (12) pp: 1541-7.

doi: 10.1016/j.joca.2012.08.026.

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Chapter 8

Cross-sectional and longitudinal associations between circulating leptin and knee cartilage thickness in older adults

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http://ard.bmj.com/content/early/2013/09/27/annrheumdis-2013-203308.abstract

Oliver P Stannus, Yuelong Cao, Benny Antony, Leigh Blizzard, Flavia Cicuttini, Graeme Jones, Changhai Ding. 2013. Cross-sectional and longitudinal associations between circulating leptin and knee cartilage thickness in older adults. Annals of the Rheumatic Diseases. Published Online First 27 September 2013.

doi:10.1136/annrheumdis-2013-203308

Chapter 9 - A pilot study on cartilage signal intensity on T1 weighted MRI: association with risk factors and measures of knee osteoarthritis

9.1 Introduction

OA is a common disease affecting older adults, with prevalence of about 25% for both symptomatic and radiographic OA in persons 55 years of age or older [192, 283]. Many major OA risk factors have been identified, including age [9], being female [15], obesity [4], and genetic factors [20]. Despite high prevalence and huge burden [284] of the disease, there are currently no approved disease-modifying anti-OA drugs (DMOADs). Discoveries for DMOADs have been impeded by lacking of sensitive tools to measure disease progression of OA, and this has stimulated research into biomarkers of OA pathology [285]. A major change in early OA is believed to be an imbalance in cartilage homeostasis [286], characterised by overactive catabolic activity and reduced anabolic activity in cartilage matrix metabolism. Several biochemical markers of early OA have been investigated. Biomarkers such as urinary C-telopeptide of type II collagen (uCTX-II) [287], and cartilage oligometric matrix protein (COMP) [288], have shown some prognostic utility but lack of specificity. The OA research community is still searching for new markers for early OA.

MRI is a potential tool in assessing early joint changes of OA. MRI has enabled the assessment of common morphological characteristics of OA not otherwise assessable, including cartilage volume, cartilage defects and bone marrow lesions. Much of current cartilage defect grading schemes are devoted to describing later stage cartilage breakdown, up to complete denuding of cartilage; similarly, cartilage volume, a common measure of OA progression, is limited by its inability to describe early pathological changes. Such pre-clinical cartilage degradation may be best defined by variations in MRI signal intensity rather than morphological features [248, 289, 290].

A recent trend in OA research is the investigation of changes in cartilage with various MR imaging modalities. These include T2 relaxation time mapping, T1-rho and invasive contrast enhanced imaging such as dGEMRIC [291], all of which require special and expensive MRI sequences. There is some preliminary evidence from investigations using 0.18T MRI that T1 signal changes in cartilage represent an early loss of defined cartilage sub-structure [105]. Studying these early cartilage changes may allow the creation of new measures of early OA using an existing and commonly used MRI modality.

The aim of this study, therefore, was to develop software to measure mean signal intensity on T1-weighted MRI in regions of articular knee cartilage and investigate the

associations between this measure and known risk factors and radiographic and MRI measures of OA in samples of both younger and older adults.

9.2 Materials and methods

9.2.1 Subjects

This study used a cross-sectional sample of 50 younger adult subjects from the KCV study, as described in section 3.3.1. These subjects were the first 50 subjects enrolled who had MRIs taken.

A second portion of the study used 168 subjects from the TASOAC study, as described in section 3.2.1. These subjects were those who had had baseline and followup serum biomarkers and MRI measures performed.

9.2.2 Magnetic resonance imaging

T1 and T2-weighted MRI scans were obtained at baseline and first followup for the older adults as described in section 3.2.8, and for the younger adults at their baseline timepoint as described in section 3.3.3. Images were checked for image noise and structural abnormalities interfering with segmentation.

Semi-automated cartilage segmentation was in all these scans, as described in sections 3.2.14 and 3.3.6, and measures of mean T1 signal intensity for regions of cartilage were determined as described in sections 3.2.15 and 3.3.7.

9.2.3 Cartilage defects

Cartilage defects were assessed for each compartment, using a 5-point scale as described in section 3.2.10.

9.2.4 Image segmentation

Semi-automated cartilage segmentation was performed in subjects at baseline and followup, as described in section 3.2.14

9.2.5 MRI measures

Measures of mean cartilage T1 signal intensity for regions of cartilage were determined as described in section 3.2.16.

9.2.6 Anthropometrics

Height, weight and derived BMI were obtained as described in section 3.2.3.

9.2.7 X-ray

Knee radiographs were taken and right knees were assessed for JSN and osteophytes as described in 3.2.5. Prevalence of joint space narrowing or osteophytes was defined respectively as any narrowing or osteophytes with grade ≥1 in each of the medial or lateral tibiofemoral compartment. ROA was defined as prevalent joint space narrowing or osteophytes in either compartment.

9.2.8 Urinary CTX-II

Urinary levels of CTX-II were measured as described in section 3.3.5.

9.2.9 Statistics

Mean signal intensity was treated as the outcome in all cross-sectional regression analyses, and the predictor in longitudinal analyses. Univariable and multivariable linear regressions were used to examine the cross-sectional associations in both study groups of both cartilage defect scores and BMI with mean signal intensity of cartilage in each compartment, before and after adjustment for age, sex, prevalent ROA, and BMI or prevalent cartilage defects. Additionally, the cross-sectional associations between CTX-II and mean signal intensity were examined in the younger adult group, both before and after adjustment for above covariates. The cross-sectional associations between ROA and mean signal intensity were examined similarly in the older adult group, where medial, lateral and total (summed over medial and lateral compartments) tibiofemoral JSN scores and medial (summed over tibia and femur), lateral (summed over tibia and femur) and combined (summed over medial and lateral compartments) osteophyte scores were analysed against intensity measures from medial, lateral or combined cartilage regions, respectively. The longitudinal associations between absolute cartilage thickness change over 2.9 years in each site and baseline measures of mean signal intensity in the same sub-region were analysed using linear regression both before and after adjustment for age, sex, BMI, ROA and cartilage defects.

A P-value less than 0.05 (two-tailed) or a 95% confidence interval not including the null point was regarded as statistically significant. All statistical analyses were performed on Stata version 10 for Windows (StataCorp, College Station, Texas, USA).

9.3 Results

The population characteristics of our sample groups are presented in Table 9.1. The prevalence of cartilage defects or radiographic OA features was low in younger adults. Due to the difference between the two groups in imaging parameters of repetition and echo time, the results were not directly comparable and the mean signal intensity of cartilage was higher in the older adults, so the subjects were analysed separately.

Table 9.1. Characteristics of participants

	Younger Adults n=50	Older Adults n = 168
Age (yrs)	40.7 (0.9)	62.6 (7.1)
Females (%)	**64**	46
BMI (kg/m²)	25.9 (0.6)	27.3 (4.2)
Prevalent Defects (%)		
Med. Fem.	2	17
Lat. Fem.	6	6
Med. Tib.	0	9
Lat. Tib.	6	4
Pat.	14	38
Prevalent JSN (%)		
Med.	12	53
Lat.	2	34
Prevalent TF osteophytes (%)		
Med.	0	7
Lat.	2	4
Mean cartilage signal intensity		
Femur	120.1 (8.4)	146.1 (10.1)
Med. Tib.	94.5 (9.8)	121.8 (8.7)
Lat. Tib.	95.9 (11.6)	123.0 (9.3)
Pat.	123.43 (10.7)	143.8 (12.6)
All	114.5 (9.3)	139.2 (9.1)

Percentages shown for proportions; otherwise: mean (SD).

Table 9.2 shows the associations in both younger and older adults between mean signal intensity of cartilage and respective cartilage defect scores in each region. In unadjusted analysis, local cartilage defects in younger adults were associated with mean signal intensity in the lateral tibial and patellar cartilage, as well as in all cartilage

combined. After adjustment for confounding variables, the first two of these associations persisted. Cartilage defect severity in older adults was negatively associated with mean signal intensity in all respective compartments, including total score with mean intensity over all cartilage. These associations persisted after adjustment for confounding variables.

The associations in both younger and older adults between BMI and measures of mean signal intensity of cartilage are presented in Table 9.3. In unadjusted analysis, BMI in younger adults was associated with reduced mean signal intensity in femoral cartilage proximal to the medial and lateral tibia and; all femoral cartilage; the medial and lateral tibial cartilage; as well as total cartilage combined. After adjustment for age, sex, prevalent ROA and prevalent local defects, all of these associations persisted (except for those in the medial tibial and total cartilage, which became of borderline significance). BMI in older adults was negatively associated with mean signal intensity in each region and all regions combined, both before and after adjustment for confounders.

Table 9.2. Associations between mean intensity measures and local cartilage defects in younger and older adults

study population	cartilage region	sub-region	univariable β (95% CI)	p	adjusted* β (95% CI)	p
younger adults						
	femoral	med. tib.	-3.58 (-11.35,4.20)	0.359	0.36 (-7.08,7.79)	0.924
		lat. tib.	-3.61 (-8.82,1.60)	0.170	-2.03 (-6.94,2.88)	0.408
	medial tibial	whole	-2.73 (-26.49,21.04)	0.819	-9.88 (-32.44,12.68)	0.382
	lateral tibial	whole	**-13.88 (-22.83,-4.94)**	**0.003**	**-10.22 (-18.54,-1.90)**	**0.017**
	patellar	whole	**-5.33 (-8.76,-1.90)**	**0.003**	**-6.06 (-9.74,-2.39)**	**0.002**
	all	whole	**-1.81 (-3.51,-0.10)**	**0.038**	-1.60 (-3.43,0.24)	0.086
older adults						
	femoral	med. tib.	**-5.16 (-7.86,-2.45)**	**<0.001**	**-4.24 (-6.63,-1.85)**	**0.001**
		lat. tib.	**-3.53 (-6.25,-0.81)**	**0.011**	**-3.35 (-5.79,-0.91)**	**0.007**
	medial tibial	whole	**-3.23 (-6.44,-0.02)**	**0.049**	**-3.58 (-6.47,-0.70)**	**0.015**
	lateral tibial	whole	**-4.50 (-7.56,-1.43)**	**0.004**	**-4.87 (-7.48,-2.26)**	**<0.001**
	patellar	whole	**-8.83 (-10.57,-7.09)**	**<0.001**	**-8.24 (-10.04,-6.44)**	**<0.001**
	all	whole	**-1.53 (-2.19,-0.86)**	**<0.001**	**-1.28 (-1.87,-0.68)**	**<0.001**

*adjusted for age, sex, BMI, and prevalent radiographic OA. CI: confidence interval; BMI, body mass index; OA: osteoarthritis.

Table 9.3. Associations between mean intensity measures and BMI in younger and older adults

study population	cartilage region	sub-region	univariable β (95% CI)	p	adjusted* β (95% CI)	p
younger adults						
	Femoral	med. tib.	**-0.96 (-1.56,-0.36)**	**0.002**	**-0.87 (-1.44,-0.30)**	**0.003**
		lat. tib.	**-0.83 (-1.45,-0.20)**	**0.010**	**-0.75 (-1.34,-0.15)**	**0.015**
		pat.	-0.52 (-1.18,0.13)	0.114	-0.45 (-1.12,0.21)	0.176
		whole	**-0.69 (-1.25,-0.13)**	**0.017**	**-0.69 (-1.25,-0.12)**	**0.019**
	medial tibial	whole	**-0.67 (-1.34,0.00)**	**0.049**	-0.55 (-1.20,0.10)	0.096
	lateral tibial	whole	**-0.99 (-1.76,-0.22)**	**0.013**	**-0.83 (-1.53,-0.13)**	**0.021**
	Patellar	whole	-0.66 (-1.39,0.07)	0.075	-0.38 (-1.13,0.37)	0.318
	All	whole	**-0.70 (-1.33,-0.08)**	**0.028**	-0.54 (-1.16,0.08)	0.084
older adults						
	Femoral	med. tib.	**-1.41 (-1.76,-1.06)**	**<0.001**	**-1.31 (-1.67,-0.95)**	**<0.001**
		lat. tib.	**-1.34 (-1.69,-0.99)**	**<0.001**	**-1.33 (-1.68,-0.97)**	**<0.001**
		pat.	**-1.10 (-1.48,-0.71)**	**<0.001**	**-1.11 (-1.52,-0.69)**	**<0.001**
		whole	**-1.30 (-1.62,-0.99)**	**<0.001**	**-1.24 (-1.57,-0.92)**	**<0.001**
	medial tibial	whole	**-1.14 (-1.40,-0.87)**	**<0.001**	**-1.11 (-1.40,-0.83)**	**<0.001**
	lateral tibial	whole	**-1.10 (-1.40,-0.80)**	**<0.000**	**-1.12 (-1.43,-0.82)**	**<0.001**
	Patellar	whole	**-1.18 (-1.60,-0.75)**	**<0.000**	**-0.92 (-1.34,-0.50)**	**<0.001**
	All	whole	**-1.22 (-1.50,-0.94)**	**<0.000**	**-1.15 (-1.45,-0.86)**	**<0.001**

*adjusted for age, sex, prevalent radiographic OA and prevalent local defects. BMI, body mass index; CI: confidence interval; OA: osteoarthritis.

The associations in younger adults between levels of U-CTX-II and measures of mean signal intensity of cartilage are presented in Table 9.4. In unadjusted analysis, U-CTX-II levels in younger adults were negatively associated with mean signal intensity in the femoral cartilage over the medial tibia and lateral tibia, as well as total femoral cartilage. U-CTX-II was also associated with both the lateral tibial and patellar cartilage mean signal intensity, and with the mean signal intensity of total knee cartilage. After adjustment for age, sex, prevalent ROA and prevalent cartilage defects, the results for femoral and patellar cartilage remained significant. When cartilage defects of grade ≥2 were excluded, U-CTX-II was significantly associated with the mean signal intensity of total knee cartilage (β: 2.75; P: 0.021) in multivariable analyses.

Table 9.4. Associations between mean intensity measures and u-CTX-II in younger adults

cartilage region	sub-region	univariable β (95% CI)	p	adjusted* β (95% CI)	p
femoral	med. tib.	**-2.08 (-3.72,-0.45)**	**0.014**	**-2.47 (-4.32,-0.62)**	**0.010**
	lat. tib.	**-2.45 (-4.04,-0.86)**	**0.003**	**-2.04 (-3.96,-0.11)**	**0.039**
	pat.	-1.52 (-3.22,0.18)	0.078	-1.67 (-3.91,0.57)	0.141
	whole	**-1.81 (-3.27,-0.35)**	**0.016**	**-1.83 (-3.65,-0.02)**	**0.048**
medial tibial	whole	-1.69 (-3.46,0.07)	0.060	-1.17 (-3.41,1.07)	0.297
lateral tibial	whole	**-2.44 (-4.47,-0.41)**	**0.020**	-1.74 (-4.08,0.59)	0.140
patellar	whole	**-2.18 (-4.07,-0.29)**	**0.025**	**-2.58 (-5.09,-0.07)**	**0.044**
All	whole	**-1.84 (-3.47,-0.20)**	**0.029**	-1.78 (-3.79,0.23)	0.082

*adjusted for age, sex, BMI, radiographic OA and prevalent local defects. U-CTX-II: Urinary levels of C-terminal crosslinking telopeptide of type II collagen; CI: confidence interval; BMI, body mass index; OA: osteoarthritis.

The associations in older adults between tibiofemoral ROA and mean signal intensity of cartilage at respective sites are presented in Table 9.5. Medial tibiofemoral joint space narrowing score was negatively associated with mean signal intensity in the portions of femoral cartilage overlying the medial tibia, as was total joint space narrowing with mean signal intensity in whole femoral cartilage and whole knee cartilage. After adjustment for age, sex and BMI, the results remained largely unchanged (data not shown); however, after further adjustment for cartilage defects, all the associations decreased in magnitude and only association for the femoral cartilage neighbouring the medial tibia

remained significant. Similarly, compartment-specific tibiofemoral osteophyte score was negatively associated with mean signal intensity in the portions of femoral cartilage overlying the medial tibia, the lateral tibial cartilage, whole femoral cartilage and whole knee cartilage. Only the associations for femoral cartilage became non-significant after adjustment for confounding variables; in contrast, the association between lateral joint space narrowing and lateral femoral cartilage signal intensity became apparent.

Table 9.6 shows the associations between mean signal intensity and change in cartilage thickness over 2.9 years. Site-specifically, change in cartilage thickness was positively predicted by mean signal intensity in the portions of femoral cartilage overlying the medial and lateral tibia, as well as patellar cartilage and all cartilage combined. These significant associations persisted after adjustment for confounding variables, when another significant association for femoral cartilage proximal to the patella became apparent.

Table 9.5. Associations between mean intensity measures and radiographic OA in older adults

study population	cartilage region	sub-region	univariable β (95% CI)	p	adjusted* β (95% CI)	p
joint space narrowing						
	Femoral	med. tib.	**-3.93 (-6.43,-1.42)**	**0.002**	**-2.68 (-4.90,-0.46)**	**0.018**
		lat. tib.	-2.86 (-6.76,1.03)	0.149	-1.97 (-5.32,1.38)	0.247
		whole	**-2.35 (-4.17,-0.52)**	**0.012**	-1.52 (-3.13,0.08)	0.063
	medial tibial	whole	-1.01 (-3.02,1.01)	0.326	-0.03 (-1.89,1.82)	0.973
	lateral tibial	whole	-2.53 (-5.85,0.79)	0.134	-1.31 (-4.21,1.58)	0.370
	All	whole	**-1.90 (-3.57,-0.24)**	**0.025**	-1.14 (-2.61,0.33)	0.127
osteophytes						
	Femoral	med. tib.	**-5.07 (-9.86,-0.28)**	**0.038**	-2.75 (-7.15,1.65)	0.219
		lat. tib.	-5.44 (-12.33,1.45)	0.121	**-6.06 (-12.00,-0.11)**	**0.046**
		whole	**-3.89 (-7.38,-0.39)**	**0.030**	-2.97 (-6.21,0.27)	0.072
	medial tibial	whole	-2.11 (-6.51,2.30)	0.347	-0.01 (-3.56,3.54)	0.997
	lateral tibial	whole	**-6.20 (-12.17,-0.23)**	**0.042**	**-7.79 (-12.73,-2.85)**	**0.002**
	All	whole	**-3.55 (-6.73,-0.37)**	**0.029**	**-3.08 (-5.80,-0.35)**	**0.027**

*adjusted for age, sex, BMI, and prevalent local defects. OA: osteoarthritis; BMI, body mass index; CI: confidence interval.

Table 9.6. Associations between mean intensity measures and change in cartilage thickness in older adults

cartilage region	sub-region	univariable β (95% CI)	p	adjusted* β (95% CI)	p
femoral	med. tib.	**0.0047 (0.0021,0.0073)**	**<0.001**	**0.0073 (0.0041,0.0105)**	**<0.001**
	lat. tib.	**0.0040 (0.0011,0.0068)**	**0.006**	**0.0075 (0.0040,0.0110)**	**<0.001**
	pat.	0.0021 (-0.0006,0.0048)	0.128	**0.0060 (0.0026,0.0094)**	**0.001**
	whole	**0.0033 (0.0006,0.0061)**	**0.019**	**0.0065 (0.0031,0.0099)**	**<0.001**
medial tibial	whole	0.0011 (-0.0014,0.0037)	0.387	0.0027 (-0.0005,0.0059)	0.093
lateral tibial	whole	0.0017 (-0.0008,0.0042)	0.184	0.0027 (-0.0006,0.0059)	0.104
patellar	whole	**0.0019 (0.0001,0.0037)**	**0.039**	**0.0023 (0.0003,0.0043)**	**0.022**
all	whole	**0.0027 (0.0001,0.0052)**	**0.040**	**0.0048 (0.0016,0.0080)**	**0.003**

*adjusted for age, sex, BMI, radiographic OA and local cartilage defects.

9.4 Discussion

To our knowledge, this study is the first to examine the relationships between inter-subject variation in T1 mean signal intensity and known OA risk factors. We found that increased BMI was associated with decreased signal intensity in younger and older adults and higher levels of urinary CTX-II in younger adults were associated with lower T1 mean signal intensity in cartilage. We also found that cartilage mean signal intensity was associated with cartilage defects in both groups. Severity of joint space narrowing and osteophytes was associated with reduced mean T1 signal intensity of cartilage. Importantly, we found that mean T1 signal intensity at baseline was positively associated with absolute change in cartilage thickness over 2.9 years. These results suggest that cartilage intensity variations observed in T1-weighted MR images may reflect early osteoarthritic changes.

We used custom semi-automated segmentation software written in MATLAB to measure mean signal intensity of cartilage, as well as cartilage thickness and volume, taking on average about 29 minutes, which is far less than manual measurement of cartilage volume (>2 hours for whole knee). We did not focus on cartilage volume in this study, as cartilage volume has been well researched and is not regarded as an early marker of OA [177].

OA has recently been described as a disease of four ordered stages [292]: first, early molecular changes occur in cartilage and joint tissue; second, structural changes visible on MRI; third, structural changes which are radiographically evident; and a final-stage characterised by joint death and replacement. Currently, OA measurement focusses on disease features in third stage disease; radiographic measures are the oldest and most widely accepted markers of OA. X-ray grades such as the Kellgren–Lawrence score [82] using joint space narrowing and osteophytes are useful for providing a clear definition of established OA in individuals and monitoring the prevalence and long-term incidence of disease in the community. We found associations between both joint space narrowing and osteophytes and reduced mean T1 signal intensity of cartilage at various sites, independent of age, sex and BMI. However, after adjustment for local cartilage defects, the associations for JSN decreased in magnitude, suggesting that the associations may be in part mediated by cartilage defects. However, the associations between mean cartilage signal intensity and osteophytes were largely independent of all confounders including cartilage defects. These results support criterion validity of mean cartilage signal intensity measurement.

More developments in OA quantification include quantitative scoring systems which assign absolute grades to various disease features [113, 293, 294]. These MRI-based measurements give sensitivity to quantify even earlier pre-radiographic changes, based on a variety of cartilage and other tissue abnormalities. Knee cartilage defects are a well-studied early-stage OA feature which can be graded visually and directly from MRI images, and are associated with later-stage features such as cartilage volume loss [242] and radiographic OA [96], as well as knee pain [194]. We found that cartilage defects were associated with lower T1-weighted mean signal intensity of cartilage, and this relationship was statistically significant for the lateral tibial and patellar portions of cartilage in younger adults and at all sites in older adults. These results suggest that our measure is including some contribution of cartilage defects towards a loss of compartment-specific image intensity, which is to be expected, given that cartilage defects on T1-weighted MRI include portions of cartilage with visibly lower signal intensity. However, smaller or more diffuse changes in signal intensity may predate the appearance of obvious cartilage defects, i.e. appear in an earlier stage of OA. These results lend face validity to the idea that early cartilage damage may be detectable on conventional T1-weighted MRI.

These associations with radiographic OA and cartilage defects suggest that loss of mean T1 signal intensity in cartilage may be a feature of OA pathophysiology prior to defects, volume loss and joint space narrowing or osteophytes. Recently, MRI has been applied to detect OA changes in cartilage before even the appearance of focal defects, and various measures of cartilage health have been derived from MRI. Average T2 relaxation time calculated from multiple T2 images is one measure used in studies of knee cartilage in OA, and has been shown to increase with radiographic severity [295]; also, symptomatic OA appears to be characterised by an increase in mean T2 relaxation time specifically in the more superficial portions of knee cartilage [296]. Similarly, delayed gadolinium-enhanced MRI of Cartilage (dGEMRIC) provides a measure of cartilage degradation reflected by loss of glycosaminoglycan (GAG) concentration [103, 104]. The measurement of T1ρ relaxation times of cartilage is another MRI-based technique used to study cartilage degeneration, sensitive to proteoglycan depletion [297]. Despite these investigations in various modalities, few papers have investigated T1-weighted signal intensity measures in OA research. Some studies reported that cartilage homogeneity on T1 weighted MRI could be a prognostic/diagnostic marker of radiographic OA or knee pain [14, 29, 30], but the relationships between homogeneity and other OA outcomes or risk factors are not known.

Changes in biochemical biomarkers can reflect the earliest features of OA. As changes in T1 MRI signal intensity may reflect cartilage changes prior to other MRI features such as established cartilage defects or volume loss, we compared our measure with U-CTX-II as a measure of early cartilage breakdown [298]. Of the various established biomarkers in OA research, CTX-II is acknowledged as a leading biomarker of early OA, despite the ambiguity of its exact tissue origin [285].CTX-II is associated with cartilage defects [96], severity of radiographic OA [299], loss of cartilage volume [300] and knee pain [301]. We found negative associations between T1-weighted mean signal intensity of cartilage and CTX-II, after adjustment for disease features and other variables. Our results suggest that signal abnormalities on T1-weighted MRI are associated with early cartilage biochemical changes, particularly in those without evident morphological changes.

To add to the construct validity of our measure in OA aetiology, we tested the associations between mean T1 signal intensity and BMI. BMI is a notable risk factor for knee OA, and is associated with cartilage defects [96] and their progression [93]. BMI is not associated with knee cartilage volume in relatively young healthy subjects [116] but predicts cartilage volume loss among those with high baseline cartilage volume in this cohort suggesting BMI can induce cartilage loss in the early stage when cartilage swelling appears [302]. We found that those with higher BMI had lower T1-weighted mean signal intensity of cartilage in various compartments in both younger and older adults. This association was independent of age, sex, cartilage defects and R OA, suggesting BMI may induce signal intensity changes in cartilage in the very early stages of OA when biochemical changes are evident.

To test the predictive validity of our measure, we examined the associations of mean cartilage T1 signal intensity with cartilage loss over time, as measured by changes in regional cartilage thickness. Loss of cartilage thickness has been used increasingly to measure cartilage loss and may represent earlier-stage change of OA than loss of cartilage volume [303]. We found that baseline cartilage signal intensity was positively associated with change in cartilage thickness, site-specifically, which was independent of age, sex, BMI, cartilage defects and ROA. This result suggests that lower cartilage signal intensity can predict cartilage loss over time.

Our study used both relatively young and older cohorts, and the results were reproduced, suggesting low T1-weighted signal intensity of cartilage appears to reflect early cartilage changes. It is not clear what could cause relatively low T1-weighted mean signal intensity of cartilage in early degradation. Theoretically, a shift in the cartilage

tissue towards a longer T1 relaxation time, or a loss of overall proton density could explain this result. Such an effect may be consistent with the concept of an increase in homogeneity [290], such as through a signal drop in the most signal-intense regions of healthy cartilage an overall drop in signal throughout the cartilage, which is suggested may reflect higher levels of fluid increased water content [16]. Alternatively, the effect might reflect the low-signal abnormalities often associated with early-stage focal defects (i.e., grade 1) in cartilage [248]. In our analyses of mean T1 signal intensity, the associations for radiographic OA, cartilage defects and BMI were each largely unchanged after adjustment for the other two study variables, suggesting independence among the associations, however the details remain unclear.

Our study has several limitations. Firstly, the small sample size in younger adults may have limited our ability to detect potentially significant associations in some compartments. Furthermore, our study design in young adults was cross-sectional; but the longitudinal study in older adults suggests that cartilage signal intensity is predictive of reduced loss of cartilage thickness. Lastly, MRI signal intensity values may be affected by factors such as equipment, software, hardware settings, image scaling, patient size and positioning. These may explain why we found differences in signal intensity between older people and younger adults in our study. Some of these can also apply for other quantitative assessment of cartilage (e.g. cartilage volume). However, the consistent associations between cartilage signal intensity and OA risk factors or markers and particularly the finding that cartilage signal intensity predicts cartilage loss suggest the measurement of cartilage signal intensity is valid. Our preliminary results may not suggest that T1 weighted cartilage signal intensity can be used as a marker for prognosis or diagnosis of OA, but it can definitely be used to study risk factors associated with early cartilage degradation in a population with same settings. Future work could investigate the fine-scale intra-subject variation in intensity through analysis of the image texture, as has been done in other modalities [304, 305].

The strengths of our study include the selection of a relatively young representative sample of the adult community, which enabled us to perform analysis in those with early osteoarthritic changes (at the molecular stage [303]). We also used an older cohort to reproduce the findings. Another strength of this study is the use of T1 imaging, which is a widely available modality, with many pre-existing cartilage segmentation and registration solutions that may be applicable to generating T1-derived cartilage measures in research settings.

In conclusion, reduced mean signal intensity of cartilage on T1-weighted fat-supressed gradient recall echo MRI is associated with OA risk factors and predicts cartilage loss, suggesting this low cartilage signal intensity on T1-weighted MRI may reflect early osteoarthritic changes.

Chapter 10 - Summary and future directions

10.1 Summary

OA is a common disease of older adulthood which can lead to pain, disability and an overall lower quality of life. OA is considered a multifactorial disease, involving a variety of risk factors and pathological mechanisms. Injury, biochemical and biomechanical factors may contribute to early dysfunctional cartilage homeostasis, which is believed to lead cartilage defects and loss of cartilage, and are often accompanied by pain and inflammation of the joint. The end stage of OA is characterised by a catastrophic loss of cartilage leading to total loss of function. This thesis examined inflammatory, hormonal and imaging biomarkers in OA in terms of their associations with OA risk factors and measures of severity and progression. Several novel findings regarding these biomarkers were presented in this thesis, summarised below.

Chapter 4 described the associations of circulating levels of both IL-6 and leptin with hip radiographic OA in an older adult population. Serum levels of IL-6 were found to be associated with increasing severity of both axial and superior hip JSN in females. These results suggested the involvement of inflammation in hip OA. Serum levels of leptin were also found to be associated with higher risk of both superior and axial hip JSN in all subjects, after adjustment for confounders including BMI. Various associations between measures of body adiposity and hip JSN were found, and were made non-significant after adjustment for circulating leptin levels. These results suggested a link between adiposity and OA severity, possibly through metabolic pathways and independent of mechanical effects. To extend the work, a longitudinal study was required, which was carried out using knee cartilage thickness measures in Chapter 8.

Chapter 5 described the relationships between both IL-6 and TNF-α and prevalent knee radiographic OA and loss of knee cartilage. Levels of both IL-6 and TNF-α were associated with increased prevalence of medial tibiofemoral joint space narrowing in a sample including males and females, extending previous results from Chapter 4 suggesting a role for inflammatory proteins in OA. Longitudinally, baseline and change in IL-6 over 3 years both predicted loss over time of both medial and lateral tibial cartilage volume. Change in TNF-α over 3 years was also associated with loss of medial tibial cartilage volume. Furthermore, these associations for these inflammatory markers were independent of each other. While it is not clear whether the associations of OA outcomes with inflammatory markers reflect the action of local joint inflammation or a systemic pro-inflammatory environment, these results show that inflammatory pathways may be

involved in OA pathogenesis. Further study in Chapter 6 was carried out to examine whether these relationships reflected the aetiology of symptoms of OA.

Chapter 6 investigated the associations of inflammatory biomarkers and their changes over 3 years with changes in knee pain over 5 years. Baseline hs-CRP was associated with change in total knee pain and change in knee pain while sitting or while lying in bed at night. Baseline TNF-α and IL-6 were associated with change in pain while standing, and change in TNF-α was positively associated with change in total knee pain and change in pain while standing. These results showed systemic inflammation to be an independent predictor of worsening knee pain over 5 years. While the specific origin of the inflammatory factors contributing to symptoms is not clear, these results show that in addition to an apparent role in OA structural progression, inflammation may be involved in the worsening of knee pain.

Chapter 7 described the natural history of knee cartilage defects and how they predicted knee cartilage volume loss and knee joint replacement. In this study of older adults, higher grades of cartilage defects were associated with age, BMI, lateral bone size, BMLs and ROA. We found the among these older adults, the average defect score for the sample increased in all compartments over 2.9 years and very few defects regressed, quite differently from the commonly relapsing defects of younger adults [93]. Radiographic OA, tibial bone size, BMI and being female were all predictors of worsening defects. Defects themselves predicted cartilage loss over 2.9 years and joint replacement over 5 years. Overall, these findings show that cartilage defects are common in older adults and tend not to regress, and supports the idea that defects are on a causal pathway leading to cartilage loss and joint failure. This implies that defects may be a suitable target for therapeutic intervention.

Chapter 8 investigated the cross-sectional and longitudinal relationships between leptin and knee cartilage thickness in older adults, as well as the causal role of obesity in these relationships. Higher levels of leptin were cross-sectionally associated with reduced cartilage thickness at all sites. Both baseline and change in leptin levels predicted lower medial tibial cartilage thickness change. Similar associations were observed for measures of obesity relating baseline and change in cartilage thickness; however, these disappeared after adjustment for leptin. To the best of our knowledge, this was the first study to show relationships between an adipokine and cartilage loss. These results confirm and extend those in Chapter 4, suggesting that leptin may be involved in obesity-related cartilage damage in OA.

Chapter 9 described the relationships between mean signal intensity of cartilage on T1-weighted MRI and OA risk factors and outcome measures in a pilot study among both younger and older adults. Those among the younger adults with higher u-CTX-II levels had lower mean T1 signal intensity of cartilage, suggesting changes in this MRI measure could reflect the very earliest stages of cartilage degradation in OA. Lower mean T1 signal intensity in cartilage was associated with higher cartilage defect grade and higher BMI in both age groups. Older adults with lower mean signal intensity of cartilage had higher rates of joint space narrowing and osteophytes and went on to have a higher rate of cartilage thickness loss over time. These results were obtained after adjustment for potential confounding from measurements including cartilage defects. Overall, this study suggested that mean T1 signal intensity of cartilage may be a novel marker of both early OA changes and risk of cartilage loss, suggesting this may be a useful biomarker in OA, subject to more extensive investigation.

In conclusion, these series of analyses in population-based studies of adults elucidate theroles of inflammatory, metabolic, and both qualitative and quantitative imaging biomarkers in OA pathophysiology. These results provide for better detection of OA development and future progression through cartilage signal abnormalities and visible defects as well as investigative evidence for a causal or predictive role of inflammatory and metabolic mechanisms in OA, providing an understanding of novel causal pathways which may prove to be potential targets for therapeutic intervention. The following section describes the further implications and future directions of these lines of research.

10.2 Future Directions

This thesis presents several novel findings using subjects from two population-based studies of younger and older community-dwelling adults. Importantly, these studies have found preliminary evidence for the roles of metabolic and inflammatory factors in pathology of OA.

In Chapters 4 and 9, we extended previous work showing a relationship between leptin and reduced knee cartilage volume [120], describing the relationships between leptin and prevalent hip JSN and future loss of knee cartilage thickness as well as theorising a role for hormonal mediators in obesity-driven cartilage damage. These results suggest metabolic hormones may constitute a potential target for prevention of cartilage damage. Obesity is a prominent risk factor for OA, with much scope for therapeutic intervention.

Future work could extend our studies and examine the longitudinal associations between leptin and changes in hip cartilage as measured on MRI. Furthermore, our studies did not include measures of other hormones up- or down-regulated by adiposity, such as resistin, visfatin, adiponectin and ghrelin. Given the close relationships between these proteins and leptin, our results do not preclude the involvement of these other adipokines, and further investigation could be carried out into the in-vivo and in-vitro effects of all these hormones on joint structural changes and joint tissue. A closer investigation looking at the relationships between OA and the proportions of metabolically dissimilar tissues such as subcutaneous and visceral fat and infrapatellar fat pat may give further detail on which aspects of adiposity are harmful and inform research and interventions. Because leptin is tied so heavily to adipose tissue and obesity, it is difficult to determine the independent effects obesity may have through the hormone as opposed to through weight-bearing. It is not known whether humans with leptin deficiency suffer less from OA; observational studies in those with impaired leptin production, weight-matched to controls, may show if a clinically significant difference exists, as suggested by mice models of leptin insufficiency [143]. Also, ongoing research into leptin analogues for treatment of leptin deficiency may result in leptin-based therapies; it would be worthwhile to examine whether these drugs would have any effect on the development or progression of OA in a sample likely to have high leptin levels, such as obese people.

Chapters 4, 5 and 6 provide evidence for inflammatory pathways in OA and related knee pain. It is possible that the contribution of inflammatory mediators to cartilage damage and symptomatic worsening may be from tissue within or around the joint. However, our studies were somewhat limited by the use of serum measurements of inflammatory biomarkers, which are non-specific measures of the production of these compounds at any of a variety of sites in the body. Future work could repeat our analyses with measures of these markers from synovial fluid to investigate whether our observations reflect systemic or local influence. It would also be worthwhile to investigate the roles of other inflammatory cytokines such as IL-17 and IL-23 in aetiology of OA. Inflammatory pathways are a central feature of rheumatoid arthritides, and drug therapies targeting inflammatory cytokines, such as cytokine receptor antagonists, act to stop cytokines binding to receptors and up-regulating further inflammatory mediators. These therapies have not been investigated for use in OA, most likely due to their present expensive nature and the lack of clear evidence of a role of cytokines in OA progression. We have shown that the same pathways targeted in RA, such as IL-6 and TNF-α, may be involved in

progression of OA pathology and symptoms, so it may be worthwhile to study the effects in OA of biologic drugs such as infliximab and etanercept for blocking TNF-α and tocilizumab for blocking IL-6.

Chapter 7 investigated the natural history and predictive validity of cartilage defects. It is apparent from our results that the presence of cartilage defects in older adults is indicative of developing or developed OA, and defects appear to have a causal role. While the aetiology of defects remains unclear, our research shows that apart from demographic risk factors, ROA and bone size are predictors of cartilage defect progression in older adults. This suggests that abnormal bone expansion may be a contributing agent in the development of knee cartilage defects and future cartilage loss leading to joint failure. Of particular interest is our finding that cartilage defects are not only common in the older adult community, but also less likely to remit than those in younger people [93]. The difference may well represent an inability of older tissue to respond to focal damage, or may be due in part to poor distribution of biomechanical loading to due bone remodelling and diminished muscle strength and proprioception. Future work could make clear which biological processes are important in defect development and progression and suggest possible therapies for those at risk.

Chapter 9 examined further the use of MRI measures to detect early differences in cartilage, focussing in both younger and older adults on variations in signal intensity of cartilage on T1-weighted MRI, which may reflect very early changes in cartilage health equal or prior to the earliest stage of cartilage defect. Although areas of abnormally low intensity are quite common on T1-weighted MRIs of even, it is not clear what exactly these represent. Further insights may be gained by histological examination of the affected sites in cadavers. Alternatively, cross-referencing the results on standard T1 with results for the same location in other modalities such as dGEMRIC, T1ρ or T2 mapping shed further light on the significance of the observed changes. Further work is needed in larger cohorts to investigate the utility of this T1 measure in predicting incidence of clinical outcomes such as established long term cartilage loss, joint replacement or symptoms, and whether it is independent of established imaging risk factors.

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Appendices

Appendix 1 – The association between leptin, interleukin-6 and hip radiographic osteoarthritis in older people: A cross-sectional study.

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Appendix 2 – Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults.

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Appendix 2

Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults

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Appendix 3 – Associations between serum levels of inflammatory markers and change in knee pain over 5 years in older adults: a prospective cohort study.

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Appendix 3

Associations between serum levels of inflammatory markers and change in knee pain over 5 years in older adults: a prospective cohort study

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Appendix 4 – Knee cartilage defects in a sample of older adults: natural history, clinical significance and factors influencing change over 2.9 years.

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Appendix 4

Knee cartilage defects in a sample of older adults: natural history, clinical significance and factors influencing change over 2.9 years

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Appendix 5 – Cross-sectional and longitudinal associations between circulating leptin and knee cartilage thickness in older adults

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Appendix 5

Cross-sectional and longitudinal associations between circulating leptin and knee cartilage thickness in older adults

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